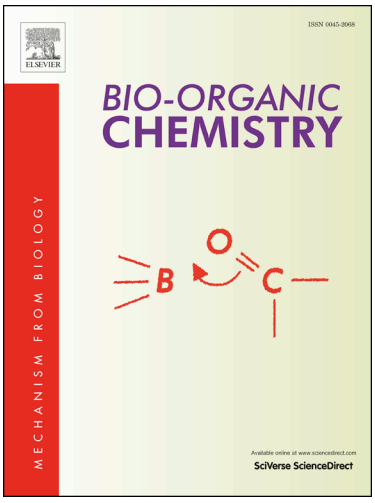

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Rationally Designed Molecules for Resurgence of Cyanide Mitigated Cytochrome c Oxidase Activity

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Abstract: Cytochrome c oxidase (CcOX) containing binuclear heme a_3-Cu B centre (BNC) mechanises the process of electron transfer in the last phase of cellular respiration. The molecular modelling based structural analysis of CcOX – heme a_3-Cu B complex was performed and the disturbance to this complex under cyanide poisoning conditions was investigated. Taking into consideration the results of molecular docking studies, new chemical entities were developed for clipping cyanide from the enzyme and restoring its normal function. It was found that the molecules obtained by combining syringaldehyde, oxindole and chrysin moieties bearing propyl / butyl spacing groups occupy the BNC region and effectively remove cyanide bound to the enzyme. The binding constant of compound **2** with CN^- was 2.3 x 10^5 M^{-1} and its ED_{50} for restoring the cyanide bound CcOX activity in 10 min was 16 µM. The compound interacted with CN^- over the pH range 5–10. The comparison of the loss of enzymatic activity in the presence of CN^- and resumption of enzymatic activity by compound **2** mediated removal of CN^- indicated the efficacy of the compound as antidote of cyanide.

Keywords: cytochrome c oxidase; cyanide; molecular modelling; design of molecules

Introduction

Cytochrome c oxidase (CcOX) is the last enzyme in the respiratory chain that takes electrons from cytochrome c (cyt c), oxidizing Fe^{2+} to Fe^{3+}, and subsequently reduces molecular oxygen to water and ultimately generates ATP. The enzyme is the life line of the living organisms; either the shortage of oxygen or the blockage of oxygen binding site of the enzyme leads to chemical asphyxiation of cells causing hypoxia that may prove lethal. Mechanistically, the working of the enzyme involves the formation of Cyt c-CcOX complex through the interaction between positively charged lysine residues on Cyt c with negatively charged residue (Glu, Asp, Tyr) on CcOX subunit-II. Active site of CcOX contains binuclear heme a_3-Cu B center (BNC) with Fe-Cu separation of 4.58 Å. Cu is coordinated to H290, H291 and H240 (Figure 1A). The imidazol ring of H240 is covalently linked to Y244 and it restricts the movements of its phenol group towards Fe site. Cu ion acts as an electron storage site and stabilizes the Fe^{2+}–O_2 adduct [1-11].

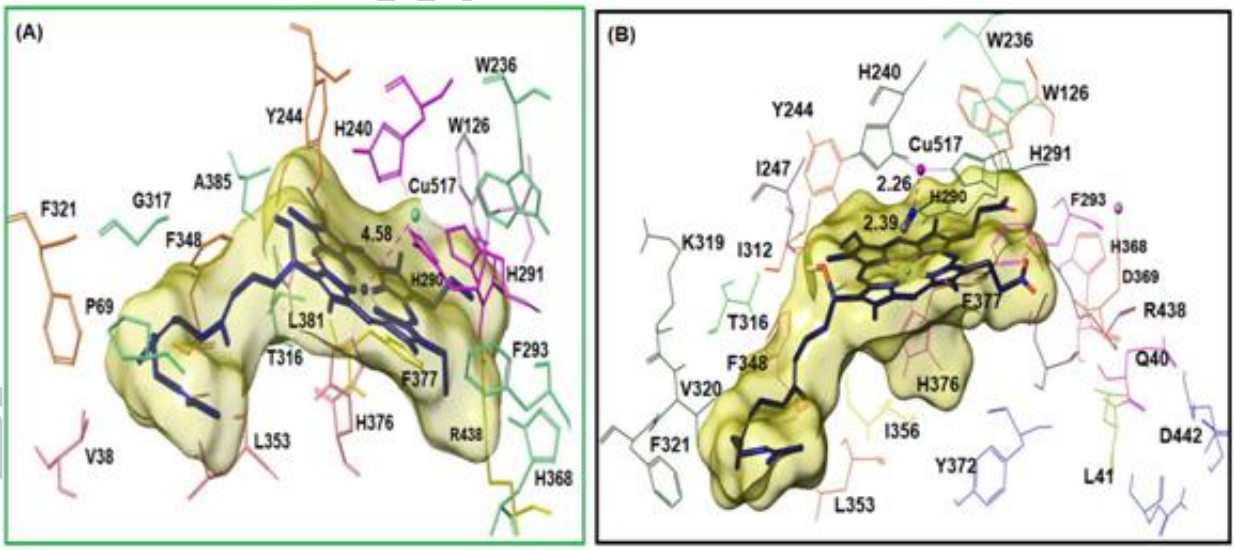


Figure 1. O_2 Binding site of CcOX showing: (A) Cu bounded to H290, H291, H240 and placed 4.58 Å from Fe and (B) cyanide ion (shown in grey-blue) is placed between Cu and Fe interacting with both the metal ions.

The cyanide mediated disturbance of the above mentioned terminal step of the cellular respiration owing to the involvement of about 1.5 million tonnes of cyanide annually in gold mining, electroplating and metallurgical process, manufacturing of perspex, nylon, acrylic plastics, nitriles, methionine and other amino acids in the animal feed industry [12-20] has far reaching physiological consequences. Cyanide targets CcOX and its toxicity [21-23] arises when this anion is sandwiched between Fe^{2+} and Cu^{+} of CcOX (Figure 1B) that results in the breakage of electron chain. The interaction of cyanide with Cu^{+} displaces H290 and the new complex competes with the molecular oxygen for binding to the catalytic site and leads to oxidative stress and limits oxidative metabolism in the body. The moment cyanide level exceeds 1.9 μM, it proves lethal [24-28]. As a remedial measure; in addition to the available reports on cyanide sensors [29-36] and cyanide scavengers [37-52] including hydroxocobalamin, dicyanocobalt (III) porphyrins, vitamin B12 analogues and hexahydrated dichlorides of cobalt (II); compound **1** (Chart 1) was also identified to remove cyanide from aqueous medium and human blood serum [53, 54]. Since chemical capturing of CN^{-} and its disposal as safe metabolite/s is desirable to get rid of cyanide poisoning, compound **1** removed cyanide through its more prevalent keto- form at the phenolic moiety and disposed it of in the form of COOH. Supporting the experimental results, the molecular docking of the keto-form of compound **1** in the oxygen/cyanide binding site of CcOX showed the approach of keto- group to cyanide at a distance 4.44 Å (Figure 2A) whereas the phenolic group in its enol- form (Figure 2B) stayed away from the CN^{-}. The molecular dynamic studies also indicated that the keto- tautomer of **1** interacted with CN^{-} maximally through its keto- group present at the phenyl ring.

In order to clip cyanide from CcOX more effectively, it was envisaged that the molecule must possess more number of C=O and OH groups so that the interactions with CN^{-} are increased. Adopting this hypothesis, we combined chrysin and compound **1** through a spacer

group to design compounds **2**–**5** expecting that oxindole and chrysin moiety may encircle the cyanide bound site of CcOX. The molecular docking of compounds **2**–**5** in the cyanide bound pocket of CcOX were compared with that of compound **1** and chrysin. It was apparent from the docking poses that the polar groups of the keto- as well as the enol- form of compound **2** (Figure 2C, 2D) were enveloping the BNC of the enzyme complex and hence it may prove as an efficient scavenger of CN^- in comparison to its precursors- compound **1** (Figure 2A) and chrysin (Figure S1). Since the non-toxicity of compound **1** has already been checked, its association with another biologically acceptable and cyanide captivating moiety chrysin may provide a new chemical entity useful as antidote of cyanide poisoning. The docking poses and interactions of compound **3** in the active site pocket of the enzyme (Figure S2) were also similar to those shown by compound **2**. However, with the increase in the length of the spacer group between chrysin and oxindole moieties, the polar groups of the resulting compounds **4** and **5** explicitly stay away from the cyanide bound BNC of the enzyme (Figure S3, S4).

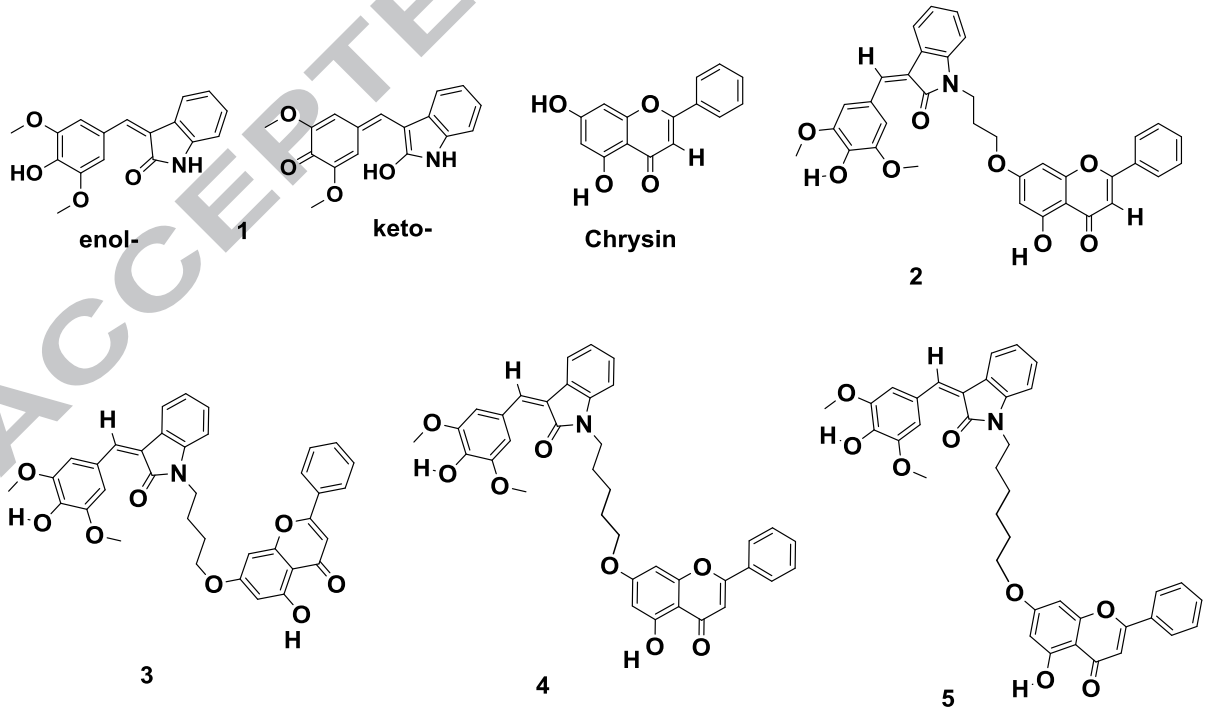


Chart 1. Cyanide scavengers reported recently (**1**) and newly designed molecules **2**-**5**.

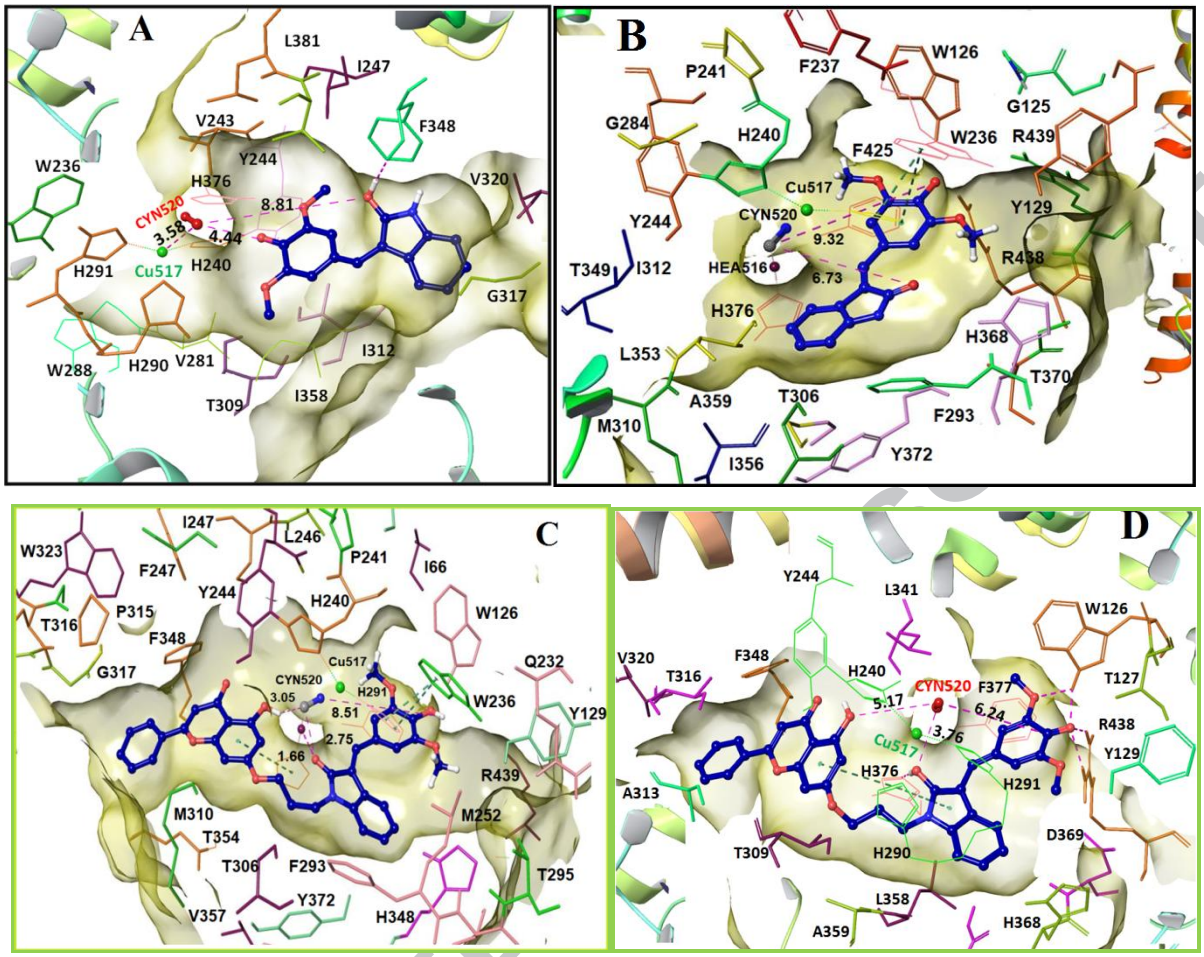
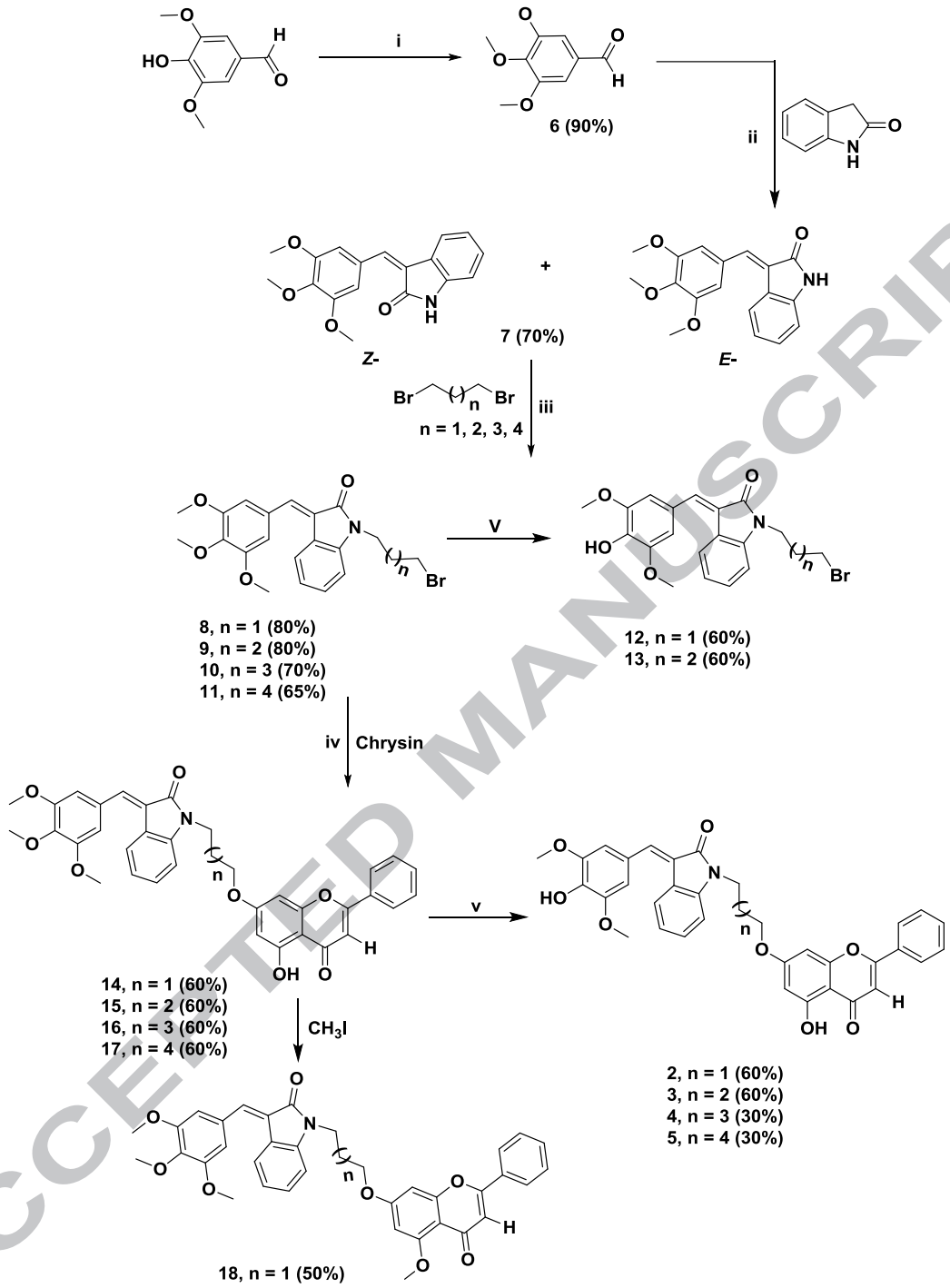


Figure 2. Compound **1** (A) keto- form and (B) enol- form docked in the oxygen binding pocket of CcOX (PDB ID 3AG4). Magenta lines show distance in Å of chrysin from cyanide (CYN520) and heme 516. Compound **2** (C) enol- form and (D) keto- form docked in CcOX. Distances of the polar groups of the molecule from cyanide and heme are represented in dotted lines. Docking of both *E*- and *Z*- isomers was tried and the molecule takes same geometry in the binding site of the enzyme.

Results and Discussion

Chemistry. The synthesis of the desired compounds was accomplished as per the protocol of Scheme 1. Compound **6**, obtained by the methylation of syringaldehyde, was made to react with oxindole by heating at 145 ^{o}C and thereby compound **7** was obtained (Scheme 1). Compound **7** was procured as *E*/*Z* isomers in the ratio 3:1. Compound **7** was treated with equivalent amount of 1,3-dibromopropane/1,4-dibromobutane/1,5-dibromopentane/1,6-dibromohexane in the presence of NaH in dimethylformamide and products **8**–**11** were



Scheme 1. Reaction conditions: i. CH_3I, DMF, K_2CO_3, rt, 6h; ii. 145 ^oC, 1h
iii. K_2CO_3, DMF, 60 ^oC, 2h; iv. K_2CO_3, DMF, 60 ^oC, 6h
v. Anhydrous $AlCl_3$, Dry DCM, N_2 atm., rt, 2h

procured. Reaction of compounds **8**–**11** with chrysin in the presence of K_2CO_3 in dimethylformamide gave respective compounds **14**–**17**. Selective demethylation of compound **14 – 17** by using anhydrous $AlCl_3$ in dry dichloromethane led to the formation of

desired compounds **2** – **5** (Scheme 1). Compounds **2** – **5** were obtained as inseparable mixture of *E*- and *Z*- isomers in the ratio 3:1 (^{1}H NMR spectrum). Compounds **12** and **13** were synthesized by the selective demethylation of **8** and **9** and these two compounds were included for making comparative studies. Methylation at the OH group of chrysin unit of compound **14** provided compound **18**.

Removal of cyanide from CcOX

Before proceeding to the enzyme immunoassays for the removal of CcOX bound cyanide, the selective interaction of compounds **2** and **3** with cyanide was ascertained. The relative change in the absorbance at 368 nm and 529 nm of the UV-vis spectra of solutions (10×10^{-6} M, DMSO:H_2O (1:9)) of compound **1**, **2**, **3**, **5** and **12** with equivalent amount of cyanide indicated that compounds **2** and **3** were more responsive to cyanide (Figure 3A, Figure S105). It seems that the length of the spacer group as in compound **5** may affect the conformation of the molecule and hence its interaction with CN^-. Addition of CN^- to 5 x 10^{-5} M solution of chrysin in DMSO:H_2O (1:9) exhibited changes in the UV-vis absorbance at 315 and 368 nm only. Both compounds **2** and **3** displayed selective binding with cyanide (Figure 3B). Stochiometry 1:2 and K_a 2.3x10^5 M^{-1} was observed for **2** and CN^- (supporting information)

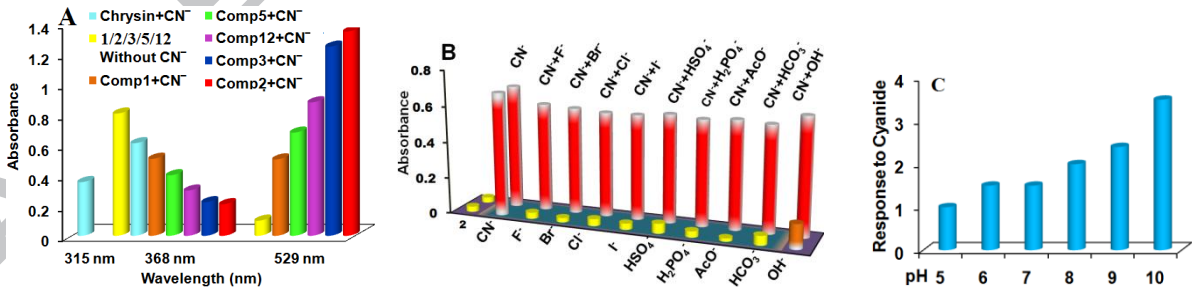


Figure 3. (A) Change in the absorbance at 368 and 529 nm of compounds **1**, **2**, **3**, **5** and **12** in the presence of CN^- (315 and 368 nm for chrysin). (B) Change in the absorbance of compound **2** at 529 nm in the presence of different anions showing selectivity and competitive binding with CN^-. (C) Response of compound **2** (change in UV-vis absorbance) to CN^- at different pH.

and this compound interacted with CN^- over pH range 5 – 10 though the effect was more pronounced at pH 9 – 10 (Figure 3C).

Cyt c-Fe^{2+} was oxidized to Fe^{3+} in the presence of CcOX and hence the absorbance intensity at 550 nm was reduced (Figure 4A). Sharp oxidative activity of CcOX attaining the saturation stage within 6 min was observed (Figure 4B). Equally fast was the diminishing in CcOX activity in the presence of cyanide and it gets lost in 8-9 min (Figure 4C, 4D). Apparently, the blockage of CcOX by cyanide is a fast process and hence need to have immediate and efficient remedial measures in case of cyanide poisoning. The stepwise

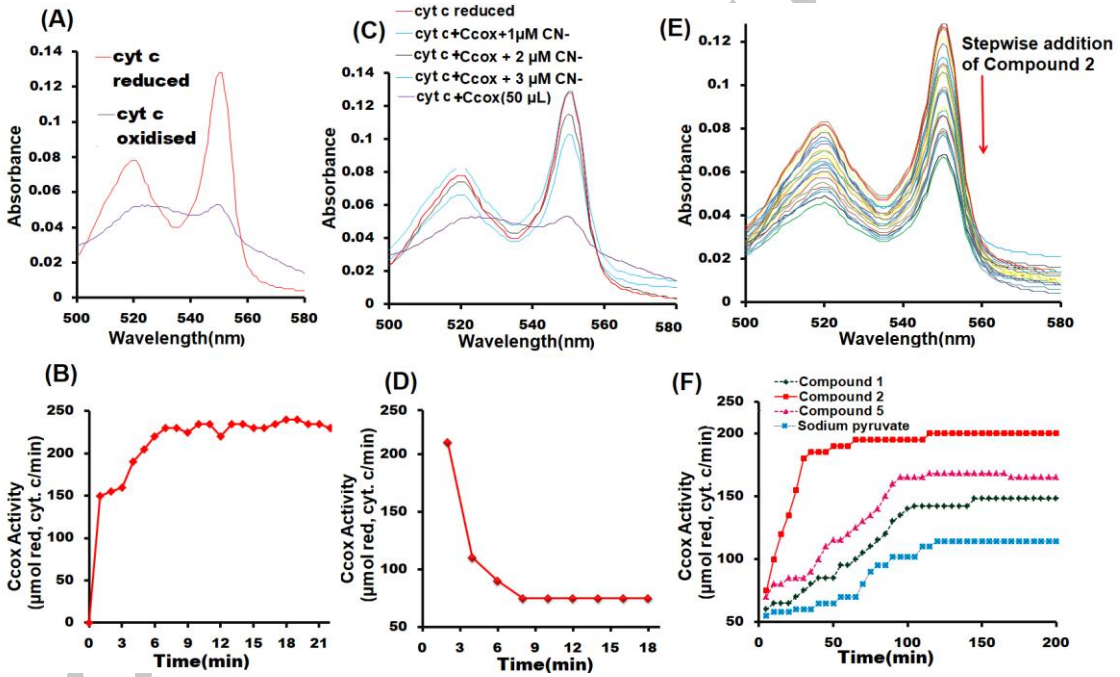


Figure 4. Working of the compound with cytochrome c oxidase: (A) UV-visible absorbance of 5.5 μM cyt c in assay buffer was monitored at wavelength 550 nm on addition of CcOX (50 μL, diluted in enzyme buffer). Decrease in absorbance was due to oxidation of Fe^{2+} to Fe^{3+} of cyt c by CcOX. (B) Activity of CcOX as a function of time. (C) Change in absorbance at 550 nm of cyt c on addition of CcOX solution along with 0 – 3 μM CN^- showing decrease in enzymatic activity of CcOX in the presence of cyanide. (D) decrease in the activity of CcOX as a function of time. (E) Absorbance intensity of solution containing cyt c, Ccox, CN^- was decreased on addition of compound **2** (1 μM to 25 μM) indicating removal of cyanide from CcOX by the compound. (F) Revival of CcOX activity by compound **1**, **2**, **5** and sodium pyruvate as a function of time.

addition of compound **2** (1 µM to 25 µM) to the above solution of cyt c – CcOX – CN^- decreased the absorbance intensity at 550 nm (Figure 4E). Apparently, 25 µM of compound **2** was sufficient to remove 3 µM cyanide from CcOX and the later oxidized cyt c-Fe^{2+} to Fe^{3+} resulting in the decrease in absorbance intensity at 550 nm. Interestingly, the resurgence of CcOX activity after the removal of CN^- by compound **2** was much fast as compared to that of compound **1**, **5** and the standard sodium pyruvate [55] (Figure 4F). The ED_{50} of compound **2** for recovering cyanide affected CcOX activity was 16 µM that was better than that of compound **1** and **5** (Table 1, Figure S108). Therefore, as per the design of the molecules, compound **2** seems an effective scavenger of CN^- capable to remove the anion from CcOX. In parallel to the results of molecular docking studies, compound **5** was less effective in the removal of CN^- from CcOX (Figure 4F).

Table 1. ED_{50} of compounds **1**, **2** and **5** for revival of CcOX activity.

compound	ED_{50} (µM)
1	30±3
2	16±2
5	27±3

Removal of cyanide from the blood serum. The results of removal of cyanide from CcOX by compound **2** were supplemented by a physical experiment. Proteinaceous blood sample was diluted in distilled water - acetone and taken in two vials marked i and ii (Figure 5). Equimolar solution of cyanide in acetone-water was added to the two vials. Compound **2** was added to vial (ii) and the solution turned red. The solutions of vial (i) and vial (ii) were extracted with ethyl acetate. The aqueous part obtained from the two solutions was treated with compound **2**. Addition of compound **2** to the aqueous part of vial (i) turned it red whereas compound **2** did not affect aqueous solution of vial (ii) indicating that all the cyanide was bound to the compound and extracted with ethyl acetate. These observations clearly

indicated that compound **2** possesses the ability to capture cyanide present in the blood serum.

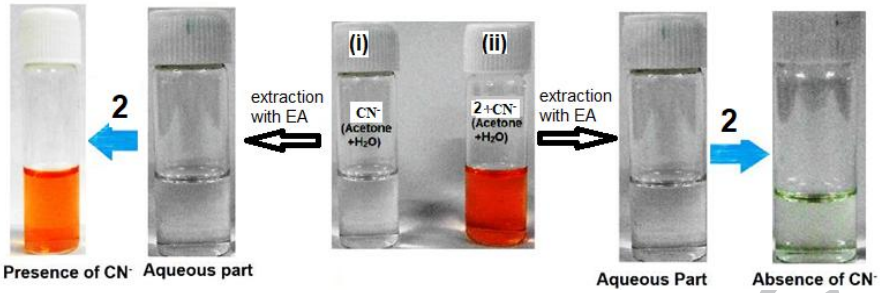
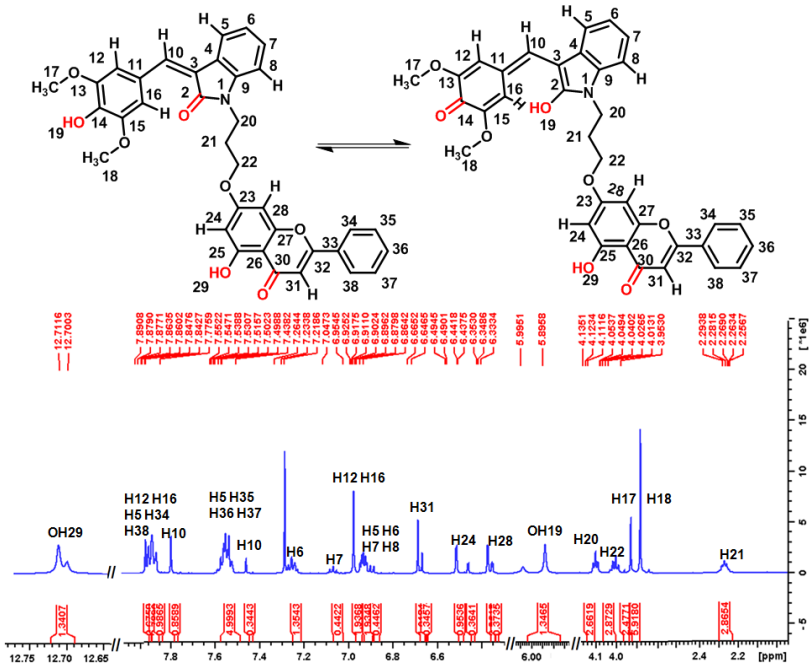


Figure 5. Schematic reperentation of the experiment showing extraction of cyanide from the human blood with the help of compound **2**.

^{1}H NMR titration experiment for confirming the probable mode of action of the compound. In consonance with the design, the part of the molecule **2** that is responsible for the interaction with CN^- was confirmed with the help of ^{1}H NMR spectra of the solution of compound and cyanide. The ^{1}H resonance assignments of the protons of compound **2** (keto- / enol- form) are depicted in Fig. 6A; the OH signals of H-19 and H-29 were assigned at δ 5.86 and 12.69, respectively (Figure 6A). The incremental addition of TBACN (0.5 equiv at each step) to the $CDCl_3$ solution of compound **2** was made and ^{1}H NMR spectra were recorded after each addition. The NMR signal corresponding to H-19 of compound **2** was finished when 1 equiv of cyanide was added (Figure 6B). The signal due to H-29 was disappeared after the addition of 3.5 equiv of cyanide. Therefore, the OH groups of compound **2** were probably responsible for capturing CN^-. Apparently, under basic pH conditions, more of the keto- form may get formed and capturing of CN^- is more effective. The participation of OH groups in capturing cyanide was also supported by the fact that compound **18**, not having free OH group, was unresponsive to CN^-. The recording of ^{1}H NMR in $CDCl_3$ by using TBACN also ruled out the possibility of deprotonation at the OH groups under the influence of hydroxide ion generated from NaCN in water.

A



B

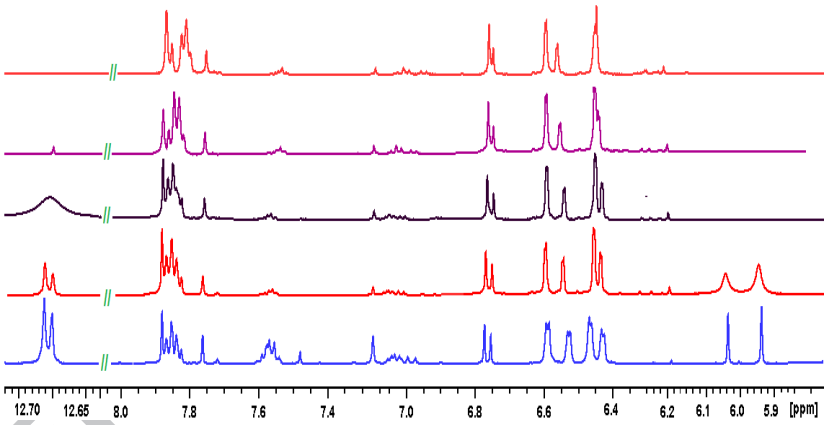


Figure 5. (A) ^{1}H NMR spectrum of compound **2**. (B) Parts of ^{1}H NMR spectra of compound **2** (8.0×10^{-3} M, 600 µL $CDCl_3$) after incremental addition of TBACN (0.5 equiv CN^- of 4×10^{-1} M in 100 µL $CHCl_3$). Peak at δ 5.89-5.99 disappear when 1 equiv CN^- was added whereas peak at δ 12.70-12.71 disappear with 3.5 equiv CN^-.

Conclusions

The molecular modelling based scrutiny of the catalytic mode of the enzyme paved the way for the rational design of new chemical entities. The modification of one of the previous molecules was made on the basis of the results of molecular docking studies and compound **2**

was found to be highly efficacious in the removal of CN^- bound to CcOX. The ED_{50} of compound **2** for reversal of CcOX activity was 16 μM and as per its design, it exhibited 1:2 stoichiometry with CN^-. This compound was not toxic to the normal cells (NCI results not shown here) and it restored the oxidizing activity of cyanide bound CcOX within 10 min. Further studies on compound **2** by using animal models are underway.

Experimental Section

^1H and ^{13}C NMR spectra were recorded on JEOL 400 MHz and Bruker 500 MHz NMR spectrometer, respectively using $CDCl_3$ as solvent. Chemical shifts are given in ppm with TMS as internal reference. Mass spectra were recorded on Bruker micrOTOF-Q II Mass spectrometer. Reactions were monitored by thin layer chromatography (TLC) on glass plates coated with silica gel GF-254. Column chromatography was performed with 60-120 mesh silica. IR and UV-vis spectral data were recorded on FTIR Agilent CARY 630 and BIOTEK Synergy H1 Hybrid Reader instruments, respectively.

Synthesis of compound 2

To the solution of compound **10** (100 mg, 0.16 mmol) in dry dichloromethane was added anhydrous $AlCl_3$ (34 mg, 0.26 mmol) under nitrogen atmosphere and the reaction mixture was stirred at room temperature for 2h. The reaction was quenched with cold water and extracted with DCM (4 × 25 mL). The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under vacuum. The crude product was purified by column chromatography using ethyl acetate/hexane (7:3) as eluent to procure a mixture of *E*- and *Z*-isomers in the ration 3:1 (^1H NMR spectrum). Yellow solid (50%), mp 175 ^oC, IR: $\bar{\upsilon}$ (cm^{-1}): 3391 (OH), 1654 (C=O), δ_H (500 MHz, $CDCl_3$): 2.25-2.29 (m, 4H, $CH_{2(major,\ minor)}$), 3.90 (s, 6H, $OCH_{3(major)}$), 3.95 (s, 6H, $OCH_{3(minor)}$), 4.01-4.05 (m, 4H, $CH_{2(major,\ minor)}$), 4.11-4.13 (m, 4H, $CH_{2(major,\ minor)}$), 5.89 (s, 1H, $OH_{(major)}$), 5.99 (s, 1H, $OH_{(minor)}$), 6.32 (d, J = 2.26 Hz, 1H, $CH_{(minor)}$), 6.34 (d, J = 2.26 Hz, 1H, $CH_{(major)}$), 6.43 (d, J = 2.26 Hz, 1H, $CH_{(minor)}$), 6.49 (d, J

= 2.26 Hz, 1H, $CH_{(major)}$), 6.64 (s, 1H, $CH_{(minor)}$), 6.66 (s, 1H, $CH_{(major)}$), 6.86 (d, *J* = 7.97 Hz, 1H, $ArH_{(minor)}$), 6.89-6.92 (m, 1H, $ArH_{(major)}$), 6.95 (s, 2H, $ArH_{(major)}$), 7.05 (t, *J* = 7.41 Hz, 1H, $ArH_{(minor)}$), 7.22 (t, *J* = 7.41 Hz, 2H, $ArH_{(major, minor)}$), 7.43 (s, 1H, bridged $H_{(minor)}$), 7.49-7.56 (m, 1H, ArH), 7.77 (s, 1H, bridged $H_{(major)}$), 7.84 (d, *J* = 2.64 Hz, 1H, $CH_{(minor)}$), 7.86 (s, 2H, $CH_{(major)}$), 7.88 (d, *J* = 7.12 Hz, 1H, $CH_{(major)}$), 12.70 (s, 1H, $OH_{(minor)}$), 12.71 (s, 1H, $OH_{(major)}$). δ_C (normal/DEPT-135; $CDCl_3$): 27.43 (CH_2), 36.86 (CH_2), 56.42 ($OCH_{3(major)}$),56.45 ($OCH_{3(minor)}$), 65.94 ($CH_{2(major)}$), 66.03 ($CH_{2(minor)}$), 93.06 ($CH_{(major}$), 93.11 ($CH_{(minor)}$), 98.67 ($CH_{(major)}$), 98.70 ($CH_{(minor)}$), 105.84 ($CH_{(minor)}$), 105.89 ($CH_{(major)}$), 105.89 (CH), 106.78 (CH), 107.81 (CH), 108.35 ($CH_{(major)}$), 109.99 ($CH_{(minor)}$), 118.54 (CH), 121.49 (CH), 121.61 (CH), 122.74 (CH), 123.41 (CH), 125.77 (C), 125.91 (C), 126.29 (C), 128.23 (CH),129.09 (CH), 129.55 (CH), 131.82 (C), 131.86 (C), 136.56 (CH), 137.85 (CH), 143.35 (C), 147.06(C), 157.71 (C), 157.78 (C), 162.15 (C), 164.65 (C), 165. 70 (C), 166.51 (C=O), 168.83 (C), 182.43 ($C=O_{(minor)}$), 182.49 ($C=O_{(major)}$). HRMS (ESI) *m/z* for $C_{35}H_{29}O_8N$ $[M+H]^+$ calcd 592.1965, found 592.2076.

Synthesis of compound 3

Anhydrous $AlCl_3$ (35 mg, 0.26 mmol) was added to the solution of compound **11** (100 mg, 0.16 mmol) in dry dichloromethane under nitrogen atmosphere and the reaction mixture was stirred at room temperature for 2h. The reaction was quenched with cold water and extracted with DCM (4 × 25 mL). The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under vacuum. The crude product was purified by chromatography using ethyl acetate/hexane (7:3) as eluent. Yellow solid (60%), mp 161 ^oC, IR: $\bar{\upsilon}$ (cm^{-1}): 3421 (OH), 1654 (C=O), δ_H (500 MHz, $CDCl_3$): 1.94 (m, 6H, $CH_{2(major)}$), 3.90 (s, 12H, $OCH_{3(major, minor)}$), 4.00 (m, 2H, $CH_{2(major)}$), 4.10 (m, 8H, $CH_{2(minor)}$), 5.86 (s, 1H, $OH_{(major)}$), 5.96 (s, 1H, $OH_{(minor)}$), 6.30 (s, 1H, $CH_{(minor)}$), 6.34 (s, 1H, $CH_{(major)}$), 6.45 (s, 1H, $CH_{(minor)}$), 6.48 (s, 1H, $CH_{(major)}$), 6.85 (d, *J* = 7.37 Hz, 1H, $ArH_{(minor)}$), 6.89 (d, *J* = 3.15 Hz, 1H, $ArH_{(major)}$), 6.91 (t, *J* = 3.09

Hz, 1H, $ArH_{(major)}$), 6.94 (s, 2H, $ArH_{(major)}$), 7.06 (t, *J* = 7.90 Hz, 1H, $ArH_{(minor)}$), 7.42 (s, 1H, bridged $H_{(minor)}$), 7.51-7.56 (m, 5H, ArH), 7.77 (s, 1H, bridged $H_{(major)}$), 7.84 (d, *J*=7.97 Hz, 2H, $CH_{(major,\ minor)}$), 7.87 (d, *J*=7.97 Hz, 2H, $CH_{(minor)}$), 7.92 (s, 1H, $CH_{(minor)}$), 12.68 (s, 1H, $OH_{(minor)}$), 12.69 (s, 1H, $OH_{(major)}$). δ_C (normal/DEPT-135; $CDCl_3$): 24.14 (CH_2), 26.33 (CH_2), 39.32 ($CH_{2(minor)}$), 39.47 ($CH_{2(major)}$), 56.44 ($OCH_{3(major)}$), 56.51($OCH_{3(minor)}$), 67.85 ($CH_{2(major)}$), 67.92 ($CH_{2(minor)}$), 93.05 (CH), 98.66 ($CH_{(minor)}$), 98.68 ($CH_{(major)}$), 105.73, 105.87, 106.76 (CH), 107.99 (CH), 108.50 (CH), 110.04 (CH), 118.53 (CH), 121.55 (CH), 121.67 (CH), 122.75 (CH), 123.50 (CH), 125.80 (C), 125.95 (C), 126.29 (C), 128.15 (CH), 129.08 (CH), 129.47 (CH), 131.34 (CH), 137.82 (CH), 136.52 (C), 137.82 (CH), 138.03 (CH), 143.32 (C), 146.60 (C), 147.04 (C), 157.81 (C), 162.14 ($C_{(minor)}$), 163.95 ($C_{(major)}$), 164.92 (C), 166.37 ($C{=}O_{(minor)}$), 168.78 ($C{=}O_{(major)}$), 182.46 (C=O). HRMS (ESI) *m/z* for $C_{36}H_{31}O_8N$ $[M+H]^+$ calcd 606.2122, found 606.2391.

Synthesis of compound 4

To the solution of compound **16** (100 mg, 0.16 mmol) in dry dichloromethane (DCM), anhydrous $AlCl_3$ (34 mg, 0.26 mmol) was added under nitrogen atmosphere and the reaction mixture was stirred at room temperature for 2h. The reaction was quenched with cold water and extracted with DCM (4 × 25 mL). The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under vacuum. The crude product was purified by column chromatography using ethyl acetate/hexane (7:3) as eluent to procure a mixture of *E*- and *Z*-isomers in the ratio 3:1. Yellow solid (30%), mp 180 ^oC, δ_H (500 MHz, $CDCl_3$+ DMSO-d_6): 1.73-1.82 (m, 4H, $CH_{2(major,\ minot)}$), 1.90-2.00 (m, 4H, $CH_{2(major,\ minor)}$), 2.58-2.65 (m, 4H, $CH_{2(major,\ minor)}$), 3.88 (s, 6H, $OCH_{3(major)}$), 3.96 (s, 6H, $OCH_{3\ (minor)}$), 4.07 (m, 4H, $CH_{2(major,\ minor)}$), 4.81-5.15 (m, 4H, $CH_{2(major,\ minor)}$), 5.27-5.33 (br, 1H, OH), 6.32 (s, 1H, CH), 6.47 (s, 1H, CH), 6.63 (s, 1H, CH), 6.92-6.95 (m, 2H, ArH), 7.06-7.11 (m, 1H, ArH), 7.26-7.28 (m, 1H, ArH), 7.50 (s, 1H, bridged H), 7.74 (m, 1H, ArH), 7.91-8.00 (m, 5H, ArH), 12.69 (s, 1H,

OH). δ_C (normal/DEPT-135; $CDCl_3$): 22.38 (CH_2), 23.16 (CH_2), 26.67 (CH_2), 27.10 (CH_2), 28.36 (CH_2), 31.31 (CH_2), 32.21 (CH_2), 56.29 ($OCH_{3(major)}$), 56.33 ($OCH_{3(minor)}$), 68.22 ($CH_{2(minor)}$), 68.50 ($CH_{2(major)}$), 92.99 ($CH_{(major}$), 94.32 ($CH_{(minor}$), 98.51 ($CH_{(major)}$), 98.69 ($CH_{(minor)}$), 105.51 (C), 105.68 ($CH_{(minor)}$), 106.80 ($CH_{(major)}$), 107.14 (CH), 107.30 (CH), 108.34 (CH), 109.91 (CH), 110.50 (CH), 118.02 (CH), 121.26 (CH), 121.47 (CH), 122.45 (CH), 126.25 (CH), 126.44 (C), 129.07 (CH), 129.26 (CH), 131.18 (C), 131.69 (C), 137.85 (CH), 147.38 (C), 157.77 (C), 161.92(C), 162.44 (C), 163.61 (C), 164.45 (C), 167.60 (C=O), 182.33 (C=O).

Synthesis of compound 5

To the solution of compound **17** (100 mg, 0.16 mmol) in dry dichloromethane was added anhydrous $AlCl_3$ (34 mg, 0.26 mmol) under nitrogen atmosphere and the reaction mixture was stirred at room temperature for 2h. The reaction was quenched with cold water and extracted with DCM (4 × 25 mL). The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under vacuum. The crude product was purified by column chromatography using ethyl acetate/hexane (7:3) as eluent to procure a mixture of *E*- and *Z*-isomers in the ratio 3:1. Yellow solid (30%), δ_H (500 MHz, $CDCl_3$+ DMSO-d_6): 1.14-1.29 (m, 8H, $CH_{2(major, minor)}$), 1.42-1.49 (m, 4H, $CH_{2(major, minor)}$), 1.67-1.82 (m, 4H, $CH_{2(major, minor)}$), 3.88-4.03 (s, 6H, $OCH_{3(major, minor)}$), 4.68-4.82 (m, 4H, $CH_{2(major, minor)}$), 6.33 (s, 1H, $CH_{(minor)}$), 6.47 (s, 1H, $CH_{(minor)}$), 6.63 (s, 1H, $CH_{(minor)}$), 6.65 (s, 1H, $CH_{(major)}$), 6.93-6.97 (m, 2H, ArH), 7.11 (m, 1H, ArH), 7.26 (m, 1H, ArH), 7.38-7.39 (m, 1H, ArH), 7.53 (m, 4H, ArH), 7.65-7.72 (m, 1H, ArH), 7.87-7.89 (m, 3H, ArH), 10.18 (OH), 12.69 (OH). δ_C (normal/DEPT-135; $CDCl_3$): 25.57 (CH_2), 25.70 (CH_2), 26.43 (CH_2), 28.66 (CH_2), 28.53 (CH_2), 32.24 (CH_2), 32.32 (CH_2), 34.84 (CH_2), 35.31 (CH_2), 56.38 ($OCH_{3(major)}$), 68.47 ($CH_{2(minor)}$), 70.37 ($CH_{2(major)}$), 92.97 ($CH_{(major}$), 94.28 ($CH_{(minor)}$), 98.60 ($CH_{(major)}$), 98.62 ($CH_{(minor)}$), 104.74 (C), 105.47 ($CH_{(minor)}$), 105.62 ($CH_{(major)}$), 107.03 (CH), 107.15 (CH), 110.18 (CH), 120.18 (CH),

120.97 (CH), 123.41 (CH), 125.66 (C), 126.16 (C), 129.26 (CH), 129.60 (CH), 131.33 (C), 131.77 (C), 137.55 (CH), 147.38 (C), 157.89 (C), 161.89 (C), 162.02 (C), 163.61 (C), 164.45 (C), 167.60 (C=O), 182.27 (C=O). HRMS (ESI) *m/z* for $C_{38}H_{35}O_8N$ $[M+H]^+$ calcd 634.2435, found 634.2852.

Synthesis of 3,4,5-trimethoxybenzaldehyde (6)

To the stirred solution of syringaldehyde (4 g, 21.9 mmol) in DMF (50 mL); K_2CO_3 (4.54 g, 32.92 mmol), CH_3I (3.73 g, 26.34 mmol) and KI (catalytic amount) were added. The reaction was allowed to stir overnight at room temperature. After the completion of reaction, it was quenched by adding water and extracted with ethyl acetate. The organic layer was separated, dried over Na_2SO_4, concentrated under vacuum to procure pure product **6**, creamish white solid (90%), mp 75-76 ^oC, δ_H (400 MHz; $CDCl_3$): 3.94 (s, 9H, OCH_3), 7.13 (s, 2H, ArH), 9.87 (s, 1H, CHO); δ_C (normal/DEPT-135; $CDCl_3$): 56.26 (OCH_3), 61.01 (OCH_3), 106.66 (CH), 131.64 (C), 143.49 (C), 153.57 (C), 191.10 (C=O). HRMS (ESI) *m/z* for $C_{10}H_{12}O_4$ $[M+H]^+$ calcd 197.0808, found 197.0727.

Synthesis of (*E*,*Z*)-3-(3,4,5-trimethoxybenzylidene)indolin-2-one (7)

3,4,5-Trimethoxybenzaldehyde (2 g, 10.20 mmol) and oxindole (1.35 g, 13.58 mmol) were heated at 145 ^oC for 1h. The reaction mass was purified by column chromatography to obtain compound **7** as mixture of *E*- and *Z*- isomers (3:1, ^1H NMR), yellow solid (70%), mp 156 ^oC. δ_H (500 MHz, $CDCl_3$): 3.90 (s, 6H, $OCH_{3(major)}$), 3.96 (s, 3H, $OCH_{3(major)}$), 3.96 (s, 3H, $OCH_{3(minor)}$), 3.99 (s, 6H, $OCH_{3(minor)}$), 6.89 (d, J = 7.92 Hz, 1H, $ArH_{(minor)}$), 6.91 (d, J = 2.65 Hz, 1H, $ArH_{(major)}$), 6.94 (t, J = 2.38 Hz, 1H, $ArH_{(major)}$), 6.95 (s, 2H, $ArH_{(major)}$), 7.06 (t, J = 7.90 Hz, 1H, $ArH_{(minor)}$), 7.25 (t, J = 7.38 Hz, 2H, $ArH_{(major,\ minor)}$), 7.50 (s, 1H, bridged $H_{(minor)}$), 7.54 (d, J = 7.60 Hz, 1H, $ArH_{(minor)}$), 7.78 (s, 1H, bridged $H_{(major)}$), 7.81 (d, J = 7.97 Hz, 1H, $CH_{(major)}$), 7.84 (s, 2H, $CH_{(minor)}$), 8.13 (br, 1H, $NH_{(minor)}$), 8.26 (br, 1H, $NH_{(major)}$). δ_C (normal/DEPT-135; $CDCl_3$): 56.27 (OCH_3), 61.05 (OCH_3), 106.87 (CH), 109.85 (CH),

110.16 (CH), 119.05 (CH), 121.72 (CH), 121.81 (CH), 123.18 (CH), 126.71 (C), 128.70 (CH), 129.82 (CH), 130.5 (C), 137.62 (CH), 139.57 (C), 141.53 (C), 152.79 (C), 153.31 (C), 169.52 (C=O). HRMS (ESI) *m/z* for $C_{18}H_{17}O_4N$ $[M+H]^+$ calcd 312.1230, found 312.1182.

Synthesis of compound 8

A mixture of (*E*,*Z*)-3-(3,4,5-trimethoxybenzylidene) indolin-2-one (500 mg, 1.6 mmol) was treated with 1,3-dibromopropane (486.6 mg, 2.4 mmol) in the presence of NaH (96.4 mg, 2.4 mmol) in dimethylformamide at 60 ^oC for 2h. The crude product was purified by column chromatography using ethyl acetate/hexane (1:9) as eluent. Yellow thick oil (80%), δ_H (400 MHz, $CDCl_3$): 2.26-2.33 (m, 2H, CH_2), 3.45-3.49 (m, 2H, CH_2), 3.82-3.87 (m, 2H, CH_2), 3.93 (s, 4H, OCH_3), 3.97 (s, 5H, OCH_3), 6.94 (d, J = 7.82 Hz, 1H, ArH), 7.06 (t, J = 7.53 Hz, 1H, ArH), 7.27-7.31 (m, 1H, ArH), 7.46 (s, 1H, bridged H), 7.53 (d, J = 6.99 Hz, 1H, ArH), 7.82 (s, 2H, ArH). δ_C (normal/DEPT-135; $CDCl_3$): 30.53 ($CH_{2(minor)}$), 30.68 ($CH_{2(major)}$), 30.91 ($CH_{2(minor)}$), 38.46 ($CH_{2(major)}$), 38.54 ($CH_{2(minor)}$), 56.22 ($OCH_{3(minor)}$), 56.30($OCH_{3(major)}$), 60.98 ($OCH_{3(major)}$), 61.00 ($OCH_{3(minor)}$), 106.61 (CH), 108.00 ($CH_{(major)}$), 108.47 ($CH_{(minor)}$), 109.81 ($CH_{(minor)}$), 109.90 ($CH_{(major)}$), 118.82 (CH), 121.73 (CH), 124.64 (C), 124.71 (C), 126.21 (C), 128.68 (CH), 129.82 (CH), 137.57 ($CH_{(minor)}$), 137.64 ($CH_{(major)}$), 140.51 (C), 141.13 (C), 152.69 (C), 153.24 (C), 166.18 (C=O), 168.61 (C=O).

Synthesis of compound 9

The compound **7** (500 mg, 1.6 mmol) was treated with 1,4-dibromobutane (520.5 mg, 2.4 mmol) in the presence of NaH (96.4 mg, 2.4 mmol) in dimethylformamide heated at 60 ^oC for 2h. The crude product was purified by column chromatography using ethyl acetate/hexane (1:9) as eluent. Yellow thick oil (80%), δ_H (500 MHz, $CDCl_3$): 1.91-2.00 (m, 6H, $CH_{2(major)}$), 3.47-3.51 (m, 2H, $CH_{2(major)}$), 3.84-3.87 (m, 8H, $CH_{2(minor)}$), 3.89 (s, 6H, $OCH_{3(minor)}$), 3.95 (s, 3H, $OCH_{3(major)}$), 3.95 (s, 3H, $OCH_{3(minor)}$), 3.99 (s, 6H, $OCH_{3(major)}$), 6.86 (d, J = 7.82 Hz, 1H, $ArH_{(major)}$), 6.89 (d, J = 8.01 Hz, 1H, $ArH_{(minor)}$), 6.92 (s, 1H, $ArH_{(minor)}$), 6.927-6.95 (t, J =

7.63 Hz, 1H, $ArH_{(minor)}$), 7.06-7.09 (t, *J* = 7.63 Hz, 1H, $ArH_{(major)}$), 7.29-7.31 (t, *J* = 7.42 Hz, 2H, ArH), 7.47 (s, 1H, bridged $H_{(major)}$), 7.55 (d, *J* = 7.32 Hz, 1H, $ArH_{(major)}$), 7.79 (s, 1H, bridged $H_{(minor)}$), 7.81 (d, *J* = 7.75 Hz, 1H, $ArH_{(minor)}$), 7.84 (s, 2H, $ArH_{(major)}$). δ_C (normal/DEPT-135; $CDCl_3$): 26.16 ($CH_{2(minor)}$), 26.23 ($CH_{2(major)}$), 29.79 ($CH_{2(minor)}$), 29.87 ($CH_{2(major)}$), 33.09 (CH_2), 38.80 ($CH_{2(minor)}$), 38.90 ($CH_{2(major)}$), 56.27 ($OCH_{3(minor)}$), 56.36 ($OCH_{3(major)}$), 60.96 ($OCH_{3(major)}$), 61.04 ($OCH_{3(minor)}$), 106.79 (CH), 108.02 (CH), 108.49 (CH), 110.10 (C), 118.82 (CH), 121.61 (CH), 121.74 (CH), 123.06 (CH), 124.77 (C), 124.88 (C), 126.37 (C), 128.60 (CH), 129.37 (C), 129.73 (CH), 137.47 (CH), 139.45 (C), 140.68 (C), 141.17 (C), 143.43 (C), 152.74 (C), 153.30 (C), 166.16 (C=O), 168.52 (C=O).

Synthesis of compound 10

Compound **7** (500 mg, 1.6 mmol) was treated with 1,5-dibromopentane (552 mg, 2.4 mmol) in the presence of NaH (96.4 mg, 2.4 mmol) in dimethylformamide at 60 ^oC for 2h. The crude product was purified by column chromatography using ethyl acetate-hexane (1:9) as eluent to obtain compound **10** as mixture of *E*- and *Z*- isomers (3:1, ^1H NMR). Yellow thick oil (80%), δ_H (500 MHz, $CDCl_3$): 1.51-1.58 (m, 4H, $CH_{2(major,\ minor)}$), 1.71-1.78 (m, 4H, $CH_{2(major,\ minor)}$), 1.86-1.96 (m, 4H $CH_{2(major,\ minor)}$), 3.38-3.44 (m, 4H, $CH_{2(major,\ minor)}$), 3.78-3.81 (m, 4H, $CH_{2(major,\ minor)}$), 3.87 (s, 6H, $OCH_{3(major)}$), 3.93 (s, 6H, $OCH_{3(minor)}$), 3.93 (s, 3H, $OCH_{3(minor)}$), 3.97 (s, 3H, $OCH_{3(major)}$), 6.82 (d, *J* = 7.70 Hz, 1H, $ArH_{(minor)}$), 6.85 (d, *J* = 7.70 Hz, 1H, $ArH_{(major)}$), 6.90 (s, 2H, $ArH_{(major)}$), 6.91 (t, *J* = 7.38 Hz, 1H, $ArH_{(major)}$), 7.057 (t, *J* = 7.38 Hz, 1H, $ArH_{(minor)}$), 7.26 (t, *J* = 5.73 Hz, 1H, $ArH_{(major,\ minor)}$), 7.45 (s, 1H, bridged $H_{(minor)}$), 7.53 (d, *J* = 7.54 Hz, 1H, $ArH_{(minor)}$), 7.77 (s, 1H, bridged $H_{(major)}$), 7.79 (d, *J* = 7.54 Hz, 1H, $ArH_{(major)}$), 7.83 (s, 2H, $ArH_{(minor)}$). δ_C (normal/DEPT-135; $CDCl_3$): 25.53 ($CH_{2(minor)}$), 25.57 ($CH_{2(major)}$), 26.80 ($CH_{2(major)}$), 26.82 ($CH_{2(minor)}$), 32.34 ($CH_{2(minor)}$), 32.37 ($CH_{2(major)}$), 33.36 ($CH_{2(major,\ minor)}$), 39.57 ($CH_{2(minor)}$), 39.68 ($CH_{2(major)}$), 56.26 ($OCH_{3(major)}$),

56.36 ($OCH_{3(minor)}$), 60.95 ($OCH_{3(minor)}$), 61.03 ($OCH_{3(major)}$), 106.80 (CH), 108.00 ($CH_{(major)}$), 108.47 ($CH_{(major)}$), 109.81 ($CH_{(minor)}$), 110.08 ($CH_{(major)}$), 118.79 (CH), 121.36 (C), 121.53 (CH), 121.64 (CH), 123.03 (CH), 124.77 (C), 125.00 (C), 126.46 (C), 128.54 (CH), 129.42 (C), 129.68 (CH), 130.20 (C), 137.33 ($CH_{(minor)}$), 137.35 ($CH_{(major)}$), 139.40 (C), 140.62 (C), 141.33 (C), 143.55 (C), 152.73 (C), 153.25 (C), 166.11 ($C=O_{(minor)}$), 168.46 ($C=O_{(major)}$).

Synthesis of compound 11

A mixture of **7** (500 mg, 1.6 mmol) and 1,6-dibromohexane (588.4 mg, 2.4 mmol) in dimethylformamide was heated at 60 ^{o}C for 2h in the presence of NaH (96.4 mg, 2.4 mmol). The crude product was purified by column chromatography using ethyl acetate-hexane (1:9) as eluent to obtain compound **11** as mixture of *E*- and Z- isomers (3:1, ^{1}H NMR). Yellow thick oil (70%), δ_H (500 MHz, $CDCl_3$): 1.38-1.46 (m, 4H, $CH_{2(major,\ minor)}$), 1.47-1.53 (m, 4H, $CH_{2(major,\ minor)}$), 1.70-1.76 (m, 4H, $CH_{2(major,\ minor)}$), 1.82-1.89 (m, 4H, $CH_{2(major,\ minor)}$), 3.37-3.41 (m, 4H, $CH_{2(major,\ minor)}$), 3.77-3.80 (m, 4H, $CH_{2(major,\ minor)}$), 3.87 (s, 6H, $OCH_{3(major)}$), 3.93 (s, 6H, $OCH_{3(minor)}$), 3.93 (s, 3H, $OCH_{3(minor)}$), 3.97 (s, 3H, $OCH_{3(major)}$), 6.82 (d, *J*= 7.75 Hz, 1H, $ArH_{(minor)}$), 6.85 (d, *J*= 7.75 Hz, 1H, $ArH_{(major)}$), 6.90 (s, 2H, $ArH_{(major)}$), 6.90 (t, *J*= 7.55 Hz, 1H, $ArH_{(major)}$), 7.05 (t, *J* = 7.57 Hz, 1H, $ArH_{(minor)}$), 7.26 (t, *J* = 7.78 Hz, 2H, $ArH_{(major\ +\ minor)}$), 7.45 (s, 1H, bridged $H_{(minor)}$), 7.53 (d, *J* = 7.50 Hz, 1H, $ArH_{(minor)}$), 7.77 (s, 1H, bridged $H_{(major)}$), 7.80 (d, *J* = 7.72 Hz, 1H, $ArH_{(major)}$), 7.83 (s, 2H, $ArH_{(minor)}$). δ_C (normal/DEPT-135; $CDCl_3$): 26.15 (CH_2), 27.48 (CH_2), 27.86 ($CH_{2(major)}$), 27.88 ($CH_{2(minor)}$), 32.63 (CH_2), 33.72 ($CH_{2(minor)}$), 33.75 ($CH_{2(major)}$), 39.73 ($CH_{2(minor)}$), 39.84 ($CH_{2(major)}$), 56.26 ($OCH_{3(major)}$), 56.36 ($OCH_{3(minor)}$), 60.98 ($OCH_{3(minor)}$), 61.00 ($OCH_{3(major)}$), 106.74 (CH), 108.05 ($CH_{(major)}$), 108.52 ($CH_{(minor)}$), 110.03 (CH), 118.82 (CH), 121.49 (CH), 121.61 (CH), 123.02 (CH), 124.77 (C), 125.08 (C), 126.52 (C), 128.54 (CH), 129.45 (C), 129.67 (CH), 130.24 (C), 137.30 (CH), 139.33 (C), 140.56 (C), 141.42 (C), 143.67(C), 152.73 (C), 153.28 (C), 166.11 ($C=O_{(minor)}$), 168.48 ($C=O_{(major)}$).

Synthesis of compound 12

The solution of compound **8** (100 mg, 0.23 mmol) in dry dichloromethane with addition of anhydrous $AlCl_3$ (49.55 mg, 0.37 mmol) under nitrogen atmosphere was stirred at room temperature for 2h. The reaction mass was purified by column chromatography using ethyl acetate/hexane (4:6) as eluent. Yellow thick oil (50%), δ_H (500 MHz, $CDCl_3$): 2.27-2.32 (m, 4H, $CH_{2(major,\ minor)}$), 3.46-3.49 (m, 4H, $CH_{2(major,\ minor)}$), 3.91 (s, 6H, $OCH_{3(major,\ minor}$), 3.93-3.96 (m, 4H, $CH_{2(major,\ minor)}$), 3.97 (s, 3H, $OCH_{3(minor)}$), 4.01 (s, 3H, $OCH_{3(major)}$), 5.85 (s, 1H, $OH_{(major)}$), 5.98 (s, 1H, $OH_{(minor)}$), 6.91-6.92 (d, J = 7.65 Hz, 1H, $ArH_{(major)}$), 6.94-6.956 (t, J = 7.87 Hz, 1H, $ArH_{(major)}$), 6.95 (s, 2H, $ArH_{(major)}$), 6.96 (d, J=7.87 Hz 1H, $ArH_{(minor)}$), 7.07 (t, J = 7.21 Hz, 1H, $ArH_{(minor)}$), 7.28 (t, J = 7.82 Hz, 2H, $ArH_{(major,\ minor)}$), 7.45 (s, 1H, bridged $H_{(minor)}$), 7.52 (d, J = 7.20 Hz, 1H, $ArH_{(minor)}$), 7.77 (s, 1H, bridged $H_{(major)}$), 7.83 (d, J = 7.62 Hz, 1H, $ArH_{(major)}$), 7.93 (s, 2H, $ArH_{(minor)}$). δ_C (normal/DEPT-135; $CDCl_3$): 30.51 ($CH_{2(major)}$), 30.68 ($CH_{2(minor)}$), 30.95 ($CH_{2(major)}$), 31.00 ($CH_{2(minor)}$), 38.49 ($CH_{2(minor)}$), 38.56 ($CH_{2(major)}$), 56.45 ($OCH_{3(major)}$), 56.53 ($OCH_{3(minor)}$), 106.74 ($CH_{(minor)}$), 107.94 ($CH_{(minor)}$), 108.44 ($CH_{(major)}$), 110.04 ($CH_{(major)}$), 118.54 ($CH_{(minor)}$), 121.55 ($CH_{(major)}$), 121.66 ($CH_{(minor)}$), 122.70 ($C_{(major)}$),123.35 ($C_{(minor)}$), 124.95 ($C_{(minor)}$), 125.24 ($C_{(major)}$), 125.77 ($C_{(minor)}$), 125.93 ($C_{(major)}$), 128.26 ($CH_{(minor)}$),129.56 ($CH_{(major)}$), 136.54 ($C_{(major)}$), 137.89 ($C_{(minor)}$), 138.20 (CH), 140.90 ($C_{(minor)}$), 143.25 ($C_{(major)}$), 146.63 ($C_{(minor)}$), 147.05 ($C_{(major)}$), 166.168.83 ($C{=}O_{(minor)}$), 168.78 ($C{=}O_{(major)}$).

Synthesis of compound 13

Anhydrous $AlCl_3$ (48 mg, 0.35 mmol) was added to the solution of compound **9** (100 mg, 0.22 mmol) in dry dichloromethane under nitrogen atmosphere and the reaction mixture was stirred at room temperature for 2h. The reaction mass was purified by column chromatography using ethyl acetate/hexane (4:6) as eluent. Yellow thick oil (50%), δ_H (500 MHz, $CDCl_3$): 1.90-1.96 (m, 8H, $CH_{2(major,\ minor)}$), 3.47-3.50 (m, 4H, $CH_{2(major,\ minor)}$), 3.60-

3.63 (m, 4H, $CH_{2(major,\ minor)}$), 3.82-3.86 (m, 4H, $CH_{2(major,\ minor)}$), 3.91 (s, 6H, $OCH_{3(major)}$), 4.01 (s, 6H, $OCH_{3(minor)}$), 5.86 (s, 1H, $OH_{(major)}$), 5.98 (s, 1H, $OH_{(minor)}$), 6.86 (d, J = 7.87 Hz, 1H, $ArH_{(minor)}$), 6.89 (d, J = 7.87 Hz, 1H, $ArH_{(major)}$), 6.92 (t, J = 7.87 Hz, 1H, $ArH_{(major)}$), 6.95 (s, 2H, $ArH_{(major)}$), 7.06 (t, J = 7.31 Hz, 1H, $ArH_{(minor)}$), 7.25 (t, J = 7.54 Hz 2H, $ArH_{(major,\ minor)}$), 7.44 (s, 1H, bridged $H_{(minor)}$), 7.51 (d, J = 7.81 Hz, 1H, $ArH_{(minor)}$), 7.77 (s, 1H, bridged $H_{(major)}$), 7.84 (d, J = 7.81 Hz, 1H, $ArH_{(major)}$), 7.93 (s, 2H, $ArH_{(minor)}$). δ_C (normal/DEPT-135; $CDCl_3$): 26.14 ($CH_{2(major)}$), 26.25 ($CH_{2(minor)}$), 29.74 ($CH_{2(major)}$), 29.85 ($CH_{2(minor)}$), 33.20 (CH_2), 38.78 ($CH_{2(minor)}$), 38.85 ($CH_{2(major)}$), 56.43 ($OCH_{3(major)}$), 56.50 ($OCH_{3(minor)}$), 106.67 ($CH_{(minor)}$), 107.96 ($CH_{(minor)}$), 108.48 ($CH_{(major)}$), 109.97 ($CH_{(major)}$), 118.52 ($CH_{(minor)}$), 121.55 ($CH_{(major)}$), 121.66 ($CH_{(minor)}$), 122.70 ($CH_{(major)}$), 125.31 ($C_{(major)}$), 125.93 ($C_{(minor)}$), 128.16 ($CH_{(major)}$), 129.47 ($CH_{(major)}$), 136.42 (CH), 138.05 (CH), 143.21 (C), 146.58 (C), 146.99 (C), 166.32 ($C{=}O_{(minor)}$), 168.70 ($C{=}O_{(major)}$).

Synthesis of compound 14

The solution of compound **8** (250 mg, 0.57 mmol) and chrysin (146.98 mg, 0.57 mmol) in dimethylformamide was heated at 60 ^oC for 6h in the presence of K_2CO_3 (119.9 mg, 0.84 mmol). The crude product was column chromatography using ethyl acetate/hexane (5:5) as eluent to procure a mixture of *E*- and *Z*- isomer in the ration 3:1. Yellow solid (60%), mp 182 ^oC, δ_H (500 MHz, $CDCl_3$): 2.25-2.28 (m, 4H, $CH_{2(major,\ minor}$), 3.87 (s, 6H, $OCH_{3(major,\ minor)}$), 3.91 (s, 6H, $OCH_{3(minor)}$), 3.93 (s, 6H, $OCH_{3(major)}$), 4.01-4.04 (m, 4H, $CH_{2(major,\ minor)}$), 4.10-4.13 (m, 4H, $CH_{2(major,\ minor)}$), 6.33 (d, J = 1.90 Hz, 1H, $CH_{(minor)}$), 6.35 (d, J = 1.90 Hz, 1H, $CH_{(major)}$), 6.45 (d, J = 1.90 Hz, 1H, $CH_{(minor)}$), 6.48 (d, J = 1.84 Hz, 1H, $CH_{(major)}$), 6.65 (s, 1H, $CH_{(minor)}$), 6.66 (s, 1H, $CH_{(major)}$), 6.87 (d, J = 7.86 Hz, 1H, $ArH_{(major)}$), 6.90 (t, J = 6.52 Hz, 1H, $ArH_{(major)}$), 6.91 (s, 2H, $ArH_{(major)}$), 7.04 (t, J = 8.06 Hz, 1H, $CH_{(minor)}$), 7.24 (t, J = 7.58 Hz, 2H, $ArH_{(major,\ minor)}$), 7.45 (s, 1H, bridged $H_{(minor)}$), 7.50-7.56 (m, 4H, ArH), 7.77 (s, 1H, bridged $H_{(major)}$), 7.80 (s, 2H, $ArH_{(minor)}$), 7.85-788 (m, 3H, ArH), 12.70 (s, 1H,

$OH_{(minor)}$), 12.70 (s, 1H, $OH_{(major)}$). δ_C (normal/DEPT-135; $CDCl_3$): 27.42 (CH_2), 36.81 ($CH_{2(minor)}$), 36.88 ($CH_{2(major)}$), 56.27 (OCH_3), 61.03 (OCH_3), 65.91 ($CH_{2(major)}$), 65.95 ($CH_{2(minor)}$), 93.03 ($CH_{(major)}$), 93.08 ($CH_{(minor)}$), 98.66 ($CH_{(minor)}$), 98.70 ($CH_{(major)}$), 105.78 (C), 105.88 ($CH_{(minor)}$), 105.91 ($CH_{(major)}$), 106.74 ($CH_{(minor)}$), 107.89 (CH), 108.37 (CH), 109.99 ($CH_{(major)}$), 118.82 ($CH_{(minor)}$), 121.29 (C), 121.69 (CH), 121.82 (CH), 123.07 (CH), 124.71 (C), 124.82 (C), 126.29 (CH), 128.68 (CH), 129.09 (CH), 129.35 (CH), 129.82 (CH), 131.30 (C), 131.85 (CH), 137.53 (CH), 137.58 (CH), 139.40(C), 140.60 (C), 141.27 (C), 143.51 (C), 152.69 (C), 153.29 (C), 157.73 (C), 157.79 (C), 162.15 (C), 162.17 (C), 163.95 (C), 164.01 (C), 164.63 (C), 164.67 (C), 166.34 (C=O), 168.65 (C=O), 182.42 (C=O), 182.48 (C=O). HRMS (ESI) *m/z* for $C_{36}H_{31}O_8N$ $[M+H]^+$ calcd 606.2122, found 606.6130.

Synthesis of compound 15

The solution of compound **9** (250 mg, 0.56 mmol) and chrysin (142.4 mg, 0.56 mmol) in dimethylformamide was heated at 60 ^oC for 2h in the presence of K_2CO_3 (116.195 mg, 0.84 mmol). The crude product was purified by column chromatography using ethyl acetate/hexane (5:5) as eluent to procure *E*- and *Z*- isomers in the ration 3:1. Yellow solid (60%), mp 177 ^oC, δ_H (500 MHz, $CDCl_3$): 1.92-1.94 (m, 8H, $CH_{2(major,\ minor)}$), 3.87 (s, 6H, $OCH_{3(minor)}$), 3.88-3.91 (m, 4H, $CH_{2(major,\ minor)}$), 3.92 (s, 3H, $OCH_{3(major)}$), 3.93 (s, 3H, $OCH_{3(minor)}$), 3.96 (s, 6H, $OCH_{3(major)}$), 4.08-4.12 (m, 4H, $CH_{2(major,\ minor)}$), 6.31 (d, J = 2.20 Hz,1H, $CH_{(major)}$), 6.34 (d, J = 2.20 Hz, 1H, $CH_{(minor)}$), 6.45 (d, J = 2.20 Hz,1H, $CH_{(major)}$), 6.49 (d, J = 2.20 Hz, 1H, $CH_{(minor)}$), 6.65 (s, 1H, $CH_{(major)}$), 6.66 (s, 1H, $CH_{(minor)}$), 6.85 (d, J = 7.15 Hz, 1H, $ArH_{(major)}$), 6.90 (s, 2H, $ArH_{(major)}$), 6.93 (d, J = 7.57 Hz, 1H, $ArH_{(minor)}$), 7.06 (t, J = 8.06 Hz, 1H, $ArH_{(minor)}$), 7.27 (t, J = 8.01 Hz, 2H, $ArH_{(major,\ minor)}$), 7.44 (s, 1H, bridged $H_{(major)}$), 7.49-7.56 (m, 4H, ArH), 7.78 (s, 1H, bridged $H_{(minor)}$), 7.80 (d, J = 7.89 Hz, 1H, $CH_{(minor)}$), 7.82 (s, 2H, $CH_{(major)}$), 7.86-7.89 (m, 2H, ArH), 12.68 (s, 1H, $OH_{(major)}$), 12.69 (s, 1H, $OH_{(minor)}$). δ_C (normal/DEPT-135; $CDCl_3$): 24.15 (CH_2), 26.35 (CH_2), 39.34 ($CH_{2(major)}$),

39.40 ($CH_{2(minor)}$), 56.26 ($OCH_{3(major)}$), 56.35 ($OCH_{3(minor)}$), 60.95 ($OCH_{3(major)}$), 61.03 ($OCH_{3(minor)}$), 67.85 ($CH_{2(major)}$), 65.94 ($CH_{2(minor)}$), 93.07 (CH), 98.63 ($CH_{(major)}$), 98.67 ($CH_{(minor)}$), 105.72 ($CH_{(minor)}$), 105.80 ($CH_{(major)}$), 106.82 (CH), 108.04 ($CH_{(major)}$), 108.05 ($CH_{(minor)}$), 110.10 (CH), 118.81 (CH), 121.62 (CH), 121.74 (CH), 123.07 (CH), 124.79 (C), 124.91 (C), 126.29 (C), 128.58 (C), 129.07 (CH), 129.72 (CH), 131.36 (C), 137.44 ($CH_{(major)}$), 137.48 ($CH_{(minor)}$), 139.46 (C), 140.66 (C), 144.21 (C), 152.72 (C), 153.29 (C), 157.82 (C), 162.18 (C), 163.96 (C), 164.92 (C), 166.23 ($C=O_{(major)}$), 168.52 ($C=O_{(minor)}$), 182.45 (C=O). HRMS (ESI) *m/z* for $C_{37}H_{33}O_8N$ $[M+H]^+$ calcd 620.2279, found 620.6404.

Synthesis of compound 16

Compound **10** (250 mg, 0.54 mmol) was treated with chrysin (138.02 mg, 0.54mmol) in the presence of K_2CO_3 (112.9 mg, 0.81mmol) in dimethylformamide at 60 ^oC for 6h. The crude product was purified by column chromatography using ethyl acetate/hexane (5:5) as eluent. Yellow solid (60%), mp 120 ^oC, δ_H (500 MHz, $CDCl_3$): 1.58-1.60 (m, 8H, $CH_{2(major,\ minor)}$), 1.80-1.83 (m, 4H, $CH_{2(major,\ minor)}$), 1.84-1.92 (m, 4H, $CH_{2(major,\ minor)}$), 3.86 (s, 6H, $OCH_{3(major)}$), 3.92 (s, 3H, $OCH_{3(minor)}$), 3.93 (s, 6H, $OCH_{3(minor)}$), 3.96 (s, 3H, $OCH_{3(major)}$), 4.01-4.05 (m, 4H, $CH_{2(major,\ minor)}$), 6.32 (s, 1H, $ArH_{(minor)}$), 6.34 (s, 1H, $ArH_{(major)}$), 6.45 (s, 1H, $ArH_{(minor)}$), 6.48 (s, 1H, $ArH_{(major)}$), 6.63 (s, 1H, $ArH_{(minor)}$), 6.65 (s, 1H, $ArH_{(major)}$), 6.84 (d, J = 7.85 Hz, 1H, $ArH_{(minor)}$), 6.87 (d, J = 7.85 Hz, 1H, $ArH_{(major)}$), 6.91 (t, J = 7.59 Hz, 1H, $CH_{(major)}$), 6.90 (s, 2H, $ArH_{(major)}$), 7.04 (t, J = 7.59 Hz, 1H, $CH_{(minor)}$), 7.27 (t, J = 7.81 Hz, 2H, $ArH_{(major,\ minor)}$), 7.45 (s, 1H, bridged $H_{(minor)}$), 7.50-7.54 (m, 4H, ArH), 77.78 (s, 1H, bridged $H_{(major)}$), 7.80 (d, J = 7.81 Hz, 1H, $ArH_{(major)}$), 7.82 (s, 2H, $ArH_{(minor)}$), 7.87-7.88 (m, 2H, ArH), 12.68 (s, 1H, $OH_{(major)}$), 12.74 (s, 1H, $OH_{(minor)}$). δ_C (normal/DEPT-135; $CDCl_3$): 23.43 ($CH_{2(minor)}$), 23.45($CH_{2(major)}$), 27.34 (CH_2), 28.63 ($CH_{2(major)}$), 28.68 ($CH_{2(minor)}$), 39.72 ($CH_{2(minor)}$), 39.80 ($CH_{2(major)}$), 56.28 (OCH_3), 60.97 (OCH_3), 61.05 (OCH_3), 68.30 ($CH_{2(major)}$), 68.37 ($CH_{2(minor)}$), 93.06 ($CH_{(major)}$), 93.10 ($CH_{(minor)}$), 98.68 ($CH_{(minor)}$), 99.61

($CH_{(major)}$), 105.69 (C), 105.89 (CH), 106.84 (CH), 108.05 ($CH_{(major)}$), 108.53 ($CH_{(minor)}$), 110.12 ($CH_{(major)}$), 118.81 ($CH_{(minor)}$), 121.40 (C), 121.57 (CH), 121.68 (CH), 123.07 (CH), 124.80 (C), 125.03 (C), 126.31 (CH), 126.47 (C), 128.56 (CH), 129.09 (CH), 129.42 (C), 129.70 (CH), 130.19 (C), 131.41 (C), 131.81 (CH), 137.40 (CH), 137.44 (CH), 139.46 (C), 141.39 (C), 141.27 (C), 143.63 (C), 152.75 (C), 153.31 (C), 157.84 (C), 162.18 (C), 163.98 (C), 165.08 (C), 166.19 ($C=O_{(minor)}$), 168.57 ($C=O_{(major)}$), 182.47 (C=O). HRMS (ESI) *m/z* for $C_{38}H_{35}O_8N$ $[M+H]^+$calcd 634.2435, found 634.2533.

Synthesis of compound 17

Compound **11** (250 mg, 0.53 mmol) was treated with chrysin (133.96 mg, 0.53 mmol) in the presence of K_2CO_3 (107.9 mg, 0.78 mmol) in dimethylformamide at 60 ^oC for 6h. The crude product was purified by column chromatography using ethyl acetate/hexane (5:5) as eluent. Yellow solid (50%), mp 105 ^oC , δ_H (500 MHz, $CDCl_3$): 1.47-1.55 (m, 4H, CH_2), 1.75-1.78 (m, 4H, CH_2), 1.80-1.84 (m, 4H, CH_2), 3.08-3.83 (m, 4H, $CH_{2(major,\ minor)}$), 3.86 (s, 6H, $OCH_{3(major)}$), 3.92 (s, 3H, $OCH_{3(minor)}$), 3.93 (s, 6H, $OCH_{3(minor)}$), 3.93 (s, 6H, $OCH_{3(major)}$), 4.00-4.04 (m, 2H, $CH_{2(major)}$), 4.01-4.04 (m, 2H, $CH_{2(\ minor)}$), 6.33 (s, 1H, $ArH_{(minor)}$), 6.35 (s, 1H, $ArH_{(major)}$), 6.46 (s, 1H, $ArH_{(minor)}$), 6.48 (s, 1H, $ArH_{(major)}$), 6.65 (s, 1H, CH), 6.83 (d, J = 7.83 Hz, 1H, $ArH_{(minor)}$), 6.86 (d, J = 8.01 Hz, 1H, $ArH_{(minor)}$), 6.88 (s, 2H, $ArH_{(major)}$), 6.90 (t, J = 7.72 Hz, 1H, $CH_{(major)}$), 7.04 (t, J = 7.59 Hz, 1H, $CH_{(minor)}$), 7.27 (t, J = 7.58 Hz, 2H, $ArH_{(major,\ minor)}$), 7.44 (s, 1H, bridged $H_{(minor)}$), 7.50-7.53 (m, 4H, ArH), 7.76 (s, 1H, bridged $H_{(major)}$), 7.83 (s, 2H, $ArH_{(minor)}$), 7.79 (d, J = 7.77 Hz, 1H, $ArH_{(major)}$), 7.87-78 (m, 2H, $ArH_{(minor)}$), 12.69 (s, 1H, OH). δ_C (normal/DEPT-135; $CDCl_3$): 25.74 ($CH_{2(major)}$), 25.76 ($CH_{2(major)}$), 26.62 ($CH_{2(minor)}$), 26.67 ($CH_{2(major)}$), 27.55 (CH_2), 28.81 ($CH_{2(major)}$), 28.82 ($CH_{2(minor)}$), 39.78 ($CH_{2(minor)}$), 39.83 ($CH_{2(major)}$), 56.24 (OCH_3), 56.35 (OCH_3), 60.90 (OCH_3), 61.03 (OCH_3), 68.47 ($CH_{2(major)}$), 68.49 ($CH_{2(minor)}$), 93.09 ($CH_{(major)}$), 93.11 ($CH_{(minor)}$), 98.60 ($CH_{(minor)}$), 98.63 ($CH_{(major)}$), 105.63 (C), 105.86 ($CH_{(minor)}$), 106.71

($CH_{(minor)}$), 108.06 (CH), 108.54 (CH), 110.02 ($CH_{(major)}$), 118.78 ($CH_{(minor)}$), 121.36 (C), 121.49 (CH), 121.62 (CH), 123.01 (CH), 124.77 (C), 125.07 (C), 126.29 (CH),126.49 (C), 128.54 (CH), 129.45 (CH), 129.67 (CH), 130.21 (CH), 131.38 (C), 131.80 (CH), 137.31 (CH),139.30(C), 140.53 (C), 141.43 (C), 152.71 (C), 153.26 (C), 157.80 (C), 162.14 (C), 163.92(C), 165.12 (C), 166.13 (C=O), 168.49 (C=O), 182.46 (C=O). HRMS (ESI) *m/z* for $C_{39}H_{37}O_8N$ $[M+H]^+$ calcd 648.2547, found 648.2796.

Synthesis of compound 18

To the stirred solution of compound **14** (100 mg, 0.165 mmol) in acetonitrile, K_2CO_3 (27.36 mg, 0.198 mmol), CH_3I (30 mg, 0.198 mmol) and KI (catalytic amount) were added. The reaction was allowed to stir for 6h at room temperature. After the completion of reaction, it was quenched by adding water and extracted with ethyl acetate. The organic layer was separated and dried over Na_2SO_4. The crude product was purified by column chromatography using ethyl acetate/hexane (8:2) as eluent to procure compound **18** as mixture of *E*- and *Z*-isomers in the ratio 3:1,yellow solid (60%), mp 160 ^oC, δ_H (500 MHz, $CDCl_3$): 2.27-2.31 (m, 2H, $CH_{2(major)}$), 2.27-2.31 (m, 2H, $CH_{2(minor)}$), 3.86 (s, 6H, $OCH_{3(major)}$), 3.90 (s, 6H, $OCH_{3(minor)}$), 3.932 (s, 3H, $OCH_{3(major,\ minor)}$), 3.938 (s, 3H, $OCH_{3(major)}$), 3.94 (s, 3H, $OCH_{3(minor)}$), 4.03-4.05 (m, 4H, $CH_{2(major,\ minor)}$), 4.15-4.17 (m, 4H, $CH_{2(major,\ minor)}$), 6.32 (d, J = 1.65 Hz, 1H, $ArH_{(minor)}$), 6.36 (d, J = 1.77 Hz, 1H, $ArH_{(major)}$), 6.51 (d, J = 1.85 Hz, 1H, $ArH_{(minor)}$), 6.44 (d, J=1.85 Hz, 1H, $ArH_{(major)}$), 6.66 (s, 1H, $ArH_{(minor)}$), 6.67 (s, 1H, $ArH_{(major)}$), 6.86 (d, J = 7.85 Hz, 1H, $ArH_{(minor)}$), 6.90 (s, 2H, $ArH_{(major)}$), 6.93 (t, J = 6.90 Hz, 1H, $ArH_{(major)}$), 7.04 (t, J = 7.33 Hz, 1H, $CH_{(minor)}$), 7.22 (t, J = 7.58 Hz, 2H, $ArH_{(major,\ minor)}$), 7.46 (s, 1H, bridged $H_{(minor)}$), 7.47-7.51 (m, 3H, ArH), 7.53 (d, J = 7.57 Hz, 1H, $ArH_{(minor)}$), 7.77 (s, 1H, bridged $H_{(major)}$), 7.80 (s, 2H, $ArH_{(minor)}$), 7.85-7.86 (m, 3H, ArH). δ_C (normal/DEPT-135; $CDCl_3$): 27.48 ($CH_{2(major)}$), 27.48 ($CH_{2(minor)}$), 39.92 ($CH_{2(minor)}$), 36.99 ($CH_{2(major)}$), 56.31 ($OCH_{3(major)}$), 56.34 ($OCH_{3(minor)}$), 56.45 ($OCH_{3(major,\ minor)}$), 60.97

($OCH_{3(major)}$), 61.06 ($OCH_{3(minor)}$), 65.97 ($CH_{2(major,\ minor)}$), 93.57 ($CH_{(major,\ minor)}$), 96.45 ($CH_{(minor)}$), 96.50 ($CH_{(major)}$), 106.71 (CH), 107.94 (CH), 108.41 (CH), 109.09 (CH), 109.48 (CH), 110.15 ($CH_{(major)}$), 118.82 (CH), 121.38 (C), 121.76 (CH), 121.88 (CH), 123.11 (CH), 124.79 (C), 126.00 ($CH_{(major)}$), 126.30 ($CH_{(minor)}$), 128.67 (CH), 128.96 (CH),129.31 (CH), 129.81 (CH), 130.06 (CH), 131.22 (C), 131.57 (CH), 137.65 (CH), 139.61 (C), 141.30 (C), 143.52 (C), 152.71 (C), 153.26 (C), 159.90 (C), 160.77 (C), 161.01(C), 163.14 (C), 168.73 (C=O), 177.63 (C=O).

UV-Vis titration of chrysin with CN^-

Stock solution of chrysin (5×10^{-3}M) was diluted to 50 μM concentration by using DMSO–H_2O, 1:9 v/v. Stock solution of NaCN (1×10^{-3} M, water) was prepared. The absorbance of solution was noted which shows absorption maxima at 315 nm. Incremental addition (10 μL, 1 equiv) of cyanide solution to the compound solution was made. The absorption intensity at 315 nm gets decreased and the intensity of new band at 368 nm goes up.

UV-Vis titration of compounds with CN^-

Stock solution of the compounds (1×10^{-3} M) in DMSO-H_2O (1:9) was diluted to 10 μM concentration by using H_2O. Stock solution of NaCN (1×10^{-3} M, water) was prepared. The absorbance of compound solution was noted which shows absorption maxima at 368 nm. Incremental addition (10 μL) of cyanide solution to the compound solution was made. The absorption intensity at 368 nm gets decreased and the intensity of new band at 529 nm goes up. UV-vis spectra of the compounds were also recorded in $CHCl_3$ taking TBACN as the source of CN^- (supporting information).

Selectivity and Competitive binding of CN^- with compound 2, 3

Stock solutions of CN^-, Br^-, F^-, AcO^-, $H_2PO_4^-$, HCO_3^-, CO_3^{2-}, Cl^-, I^- (10^{-3} M in water) were prepared. 200 μL (20 equiv) of each anion was added to the compound solution one by one

and UV-vis spectrum was recorded. Decrease in the absorption band at 368 nm and emergence of new band was observed only in the case of cyanide solution.

Working of the compound with cytochrome c oxidase

The preparation of assay buffer, enzyme buffer and other reagents was as per the protocol available with the assay kit [56]. The absorbance of cyt c (5.5 μM) at 550 nm was noted. To the solution of cyt c (5.5 μM having 25 μL cyt c and 975 μL assay buffer), 0.5 μL DTT (0.1 M) was added and kept for 15 min. Incremental addition (10 μL to 50 μL) of CcOX (5μL CcOX diluted with 45 μL enzyme dilution buffer) was made that led to diminishing of absorbance intensity at 550 nm. Addition of cyanide (1 μM – 3 μM) to the solution of CcOX was followed by the transfer of each of this solution (CcOX – 1 μM CN^-, CcOX – 2 μM CN^-, CcOX – 3 μM CN^-) to the vial containing cyt c and absorbance at 550 nm was increased. In order to check the affinity of compound for cyanide bound to cyt c oxidase, stepwise addition of compound **2** (1 μM to 25 μM) was made to the solution containing CcOX – 3 μM CN^- and cyt c. The absorbance intensity at 550 nm gets reduced.

Removal of cyanide from aqueous medium

Blood from the healthy subject was taken and the protocol was approved by the institute ethical committee for the use of human subjects. Cyanide (10 μL, 1×10^{-1} M) was added to each of the two vials containing 1 mL blood serum diluted in acetone-water. Compound **2** (100 μL, 1×10^{-3} M) in acetone – water (1:9, 1 mL) was added to vial ii. The solutions of two vials were extracted with ethyl acetate (4 x 25 mL). Addition of compound **2** (100 μL) to the aqueous part (obtained after extraction with ethyl acetate) of vial ii did not change the colour of the solution indicating absence of cyanide whereas the aqueous part of vial i responded to compound **2**.

Molecular modelling studies

The 3-D crystal structure of cytochrome c oxidase (CcOX) and in complex with cyanide (PDB ID 1OCC and 3AG4 respectively) were downloaded from protein data bank (www.rcsb.org) and used for the docking studies. The compounds were docked in active site of CcOX in complex with cyanide using Schrodinger (Schrödinger Release 2015-4: Maestro, version 10.0, Schrödinger, LLC, New York, NY, 2014) software package and following the protein preparation, ligand preparation, receptor grid generation steps for docking as described in one of the previous reports [57].

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ABBREVIATIONS

CcOx, cytochrome c oxidase; ED_{50}, cyto c, cytochrome c; BNC, binuclear centre; TBACN, tetrabutyl ammonium chloride; ED_{50}, effective dose causing 50% effect; ACN, acetonitrile; TLC, thin layer chromatography; DMSO, dimethyl sulphoxide; K_a, association constant.

ASSOCIATED CONTENT

Supporting information

Experimental data, ^{1}H and ^{13}C NMR spectra, mass spectra, IR spectra and molecular docking data (PDF)

References:

[1] S. Arnold. The Power of life – Cytochrome c Oxidase takes center stage in metabolic control, cell signalling and survival. Mitochondrion 12 (2012) 46-56.

[2] M. F. Lucas, D. L. Rousseau, V. Guallar. Electron Transfer Pathways in Cytochrome c Oxidase. Biochim. Biophys. Acta. 1807 (2011) 1305-1313.

[3] S. Yoshikawa, A. Shimada. Reaction Mechanism of Cytochrome c Oxidase. Chem. Rev. 115 (2015) 1936-1989.

[4] M. Denis. Structure and function of cytochrome-c oxidase. Biochimie. 68 (1986) 459-470.

[5] R. Boulatov, J. P. Collman, I. M. Shiryaeva, C. J. Sunderland. Functional Analogues of the Dioxygen Reduction Site in Cytochrome Oxidase: Mechanistic Aspects and Possible Effects of CuB. J. Am. Chem. Soc. 124 (2002) 11923-11935.

[6] W. Sato, S. Hitaoka, K. Inoue, M. Imai, T. Saio, T. Uchida, K. S.- Itoh, S. K. Yoshizawa, K. Ishimori. Energetic Mechanism of Cytochrome c- Cytochrome c Oxidase Electron Transfer Complex Formation under Turnover Conditions Revealed by Mutational Effects and Docking Simulation. J. Biol. Chem. 291 (2016) 15320-15331.

[7] K. Muramoto, K. Ohta, K. S-. Itoh, K. Kanda, M. Taniguchia, H. Nabekura, E. Yamashita, T. Tsukihara, S. Yoshikawa, Bovine cytochrome c oxidase structures enable O_2 reduction with minimization of reactive oxygens and provide a proton-pumping gate, Proc. Natl. Acad. Sci. U.S.A., 107 (2010), 7740-7745.

[8] M. R. A. Blomberg, P. E. M. Siegbahn, How cytochrome c oxidase can pump four protons per oxygen molecule at high electrochemical gradient, Biochim. Biophys. Acta, 1847 (2015), 364-376.

[9] J. A. Lyons, D. Aragão, O. Slattery, A. V. Pisliakov, T. Soulimane, M. Caffrey, Structural insights into electron transfer in caa_3-type cytochrome oxidase, Nature 487 (2012) 514-518.

[10] P. R. Rich, A. Maréchal, The mitochondrial respiratory chain, Essays Biochem. 47 (2010) 1-23.

[11] M. Assempour, B. C. Hill, Cyanide binding to different redox states of the cytochrome caa_3 complex from *Bacillus subtilis*; a member of the cytochrome oxidase super-family of enzymes, Biochim. Biophys. Acta, 1320 (1997) 175–187.

[12] R. Eisler, S. N. Wiemeyer, Cyanide Harzards to plants and animals from gold mining and related water issues. Rev. Environ. Contam. Toxicol. 183 (2004) 21-54.

[13] T. F. Cummings, The Treatment of cyanide poisoning. Occup. Med. 54 (2004) 82-85.

[14] A. R. Surleva, G. Drochioiu, Visualizing smoking hazard: A simple spectrophotometric determination of hydrogen cyanide in cigarette smoke and filters. J. Chem. Educ. 90 (2013) 1654-1657.

[15] M. A. Acheampong, R. J. W. Meulepas, P. N. L. Lens, Removal of Heavy Metals and Cyanide from Gold Mine wastewater. J. Chem. Technol. Biotechnol. 85 (2010) 590-613.

[16] F. M. Esposito, Y. Alarie, Inhalation Toxicity of Carbon Monoxide and Hydrogen Cyanide Gases released during the thermal decomposition of Polymers, J. Fire. Sci. 6 (1988) 195-242.

[17] R. R. Dash, A. Gaur, C. Balomajumder. Cyanide in industrial waste waters and its removal: A review on biotreatment. J. Hazard. Mater. 163 (2009) 1-11.

[18] J. Z. Stephan, M. P. Coughlin, M. P. Lalonde, E. N. Jacobsoen, Scaleable catalytic asymmetric strecker Syntheses of unnatural α-amino acids. Nature 461 (2009) 968-970.

[19] C. V. Vinnakota, N. S. Peetha, M. G. Perrizo, D. G. Ferris, R. P. Oda, G. A. Rockwood, B. A. Logue, Comparison of cyanide exposure markers in the bio fluids of smoker & non-smokers. Biomarkers. 17 (2012) 625-633.

[20] J. L. Way, Cyanide intoxication and its mechanism of antagonism. Annu. Rev. Pharmacol. Toxicol. 24 (1984) 451-481.

[21] D. B. Donato, O. Nichols, H. Possingham, M. Moore, P. F. Ricci, B. N. Noller, A Critical review of the effects of gold cyanide bearing tailing solutions on wildlife. Environ. Int. 33 (2007) 974-984.

[22] C. E. Cooper, G. C. Brown, The inhibition of mitochondrial cytochrome oxidase by the gases carbon monoxide, nitric oxide, hydrogen cyanide and hydrogen sulfide: chemical mechanism and physiological significance. J. Bioenerg. Biomembr. 40 (2008) 533-539.

[23] M. Panda, N. C. Robinson, Kinetics and Mechanism for the binding of HCN to cytochrome c oxidase. Biochemistry, 34 (1995) 10009-10018.

[24] L. L. Pearce, E. L. Manzano, M. -B. Sandra, J. Peterson, Antagonism of nitric oxide toward the inhibition of cytochrome c oxidase by Carbon monoxide and Cyanide, Chem. Res. Toxicol. 21 (2008) 2073-2081.

[25] *Guidelines for Drinking Water Quality*, World Health Organization, Geneva, 1996.

[26] E. Jaszczak, Ż. Polkowska, S. Narkowicz, J. Namieśnik, Cyanide in Environment-analysis-Problems and Challenges. Enviorn. Sci. Pollut. Res. 24 (2017) 15929-15948.

[27] Toxicology Profile for Cyanide. Agency for toxic substances and disease Registry; U. S. Department of Health and Human Services: Washington, D. C., 2006.

[28] Y. Tian, P. K. Dasgupta, S. B. Mahon, J. Ma, M. Brenner, J. H. Wang, G. R. Boss, A disposable blood cyanide sensor. Anal. Chim. Acta, 768 (2013) 129-135.

[29] J. Ma, P. K. Dasgupta, Recent developments in cyanide detection: A review. Anal. Chim. Acta, 673 (2010) 117-125.

[30] T. W. Hundall, F. P. Gabbai, Ammonium boranes for the selective complexation of cyanide or fluoride ions in water. J. Am. Chem. Soc. 129 (2007) 11978-11986.

[31] H. Miyaji, D. -S. Kim, B. -Y. Chang, E. Park, S. -M. Park, K. H. Ahn, Highly cooperative ion-pair recognition of potassium cyanide using a heteroditopic ferrocene-based crown ether-trifluoroacetylcarboxanilide receptor. Chem. Commun. (2008) 753-755.

[32] Y. J. Na, G. J. Park, H. Y. Jo, S. A. Lee, C. Kim, A colorimetric chemo sensor based on a Schiff base for highly selective sensing of cyanide in aqueous solution: the influence of solvents. New J. Chem. 38 (2014) 5769-5776.

[33] X. Chen, S.-W. Nam, G. -H. Kim, N. Song, Y. Jeong, I. Shin, S. K. Kim, J. Kim, S. Park, J. Yoon, A near-infrared fluorescent sensor for detection of cyanide in aqueous solution and its application for bioimaging. Chem. Commun. 46 (2010) 8953-8955.

[34] Y. Qu, B. Jin, Y. Liu, Y. Wu, L. Yang, J. Wu, J. Hua, A new triphenylamine fluorescent dye for sensing of cyanide anion in living cell. Tetrahedron Lett. 54 (2013) 4942-4944.

[35] S. Saha, A. Ghosh, P. Mahato, S. Mishra, S. K. Mishra, E. Suresh, S. Das, A. Das, Specific recognition and sensing of CN^- in sodium cyanide solution. Org. Lett. 12 (2010) 3406-3409.

[36] R. Bhattacharya, Antidotes to cyanide poisoning: Present status. Ind. J. Pharmaco. 32 (2000) 94-101.

[37] A. H. Hall, R. Dart, G. S. Bogdan, Sodium thiosulfate of hydroxocobalamin for the empiric treatment of cyanide poisoning? Ann. Emerging Med. 49 (2007) 806-813.

[38] A. H. Hall, J. Saiers, F. Baud, Which cyanide antidote? Crit. Rev. Toxicol. 39 (2009) 541-552.

[39] G. Shepherd, L. I. Velez, Role of hydroxocobalamin in acute cyanide poisoning. Ann. Pharmacother. 42 (2008) 661-669.

[40] J. P. Thompson, T. C. Marrs, Hydroxocobalamin in cyanide poisoning. Clin.Toxicol. 50 (2012) 875-885.

[41] E. L- Manzano, A. A. Cronican, K. L. Frawley, J. Peterson, L. L. Pearce. Cyanide scavenging by a Cobalt Schiff-base macrocycle: A cost effective to corrinoids. Chem. Res. Toxicol. 29 (2016) 1011-1019.

[42] V. S. Bebarta, R. L. Pitotti, P. Dixon, J. R. Lairet, A. Bush, D. A. Tanen, Hydroxocobalamin versus sodium thiosulfate for the treatment of acute cyanide toxicity in a swine (sus scrofa) model. Ann. Emerg. Med. 59 (2012) 532-539.

[43] K. Watanabe, H. Kitagishi, K. Kano, Supramolecular Ferric porphyrins as cyanide receptors in aqueous solution. ACS Med. Chem. Lett. 2 (2011) 943-947.

[44] B. Aebli, C. M. -Croiśe, F. Zelder, Controlling binding dynamics of corrin-based chemosensor for Cyanide. Inorg. Chem. 53 (2014) 2516-2520.

[45] K. E. Broderick, P. Potluri, S. Zhuang, I. E. Scheffler, V. S. Sharma, R. B. Pilz, G. R. Boss, Cyanide Detoxification by the Cobalamin Precursor Cobinamide. Exp. Biol. Med. 231 (2006) 641-649.

[46] P. Hambright, R. Langley. Cyanide scavengers: Kinetics of the reactions of cyanide with a water soluble cobalt (III) porphyrin. J. Inorg. Bio. 32 (1988) 197-205.

[47] J. Lee, S. B. Mahon, D. Mukai, T. Burney, B. S. Katebian, A. Chan, V. S. Bebarta, D.Yoon, G. R. Boss, M. Brenner, The Vitamin B_{12} Analog Cobinamide Is an Effective Antidote for Oral Cyanide Poisoning. J. Med. Toxicol. 12 (2016) 370- 379.

[48] W. W. Reenstra, W. P. Jencks, Reactions of cyanide with cobalamins. J. Am. Chem. Soc. 101 (1979) 5780-5791.

[49] O. S. Benz, Q. Yuan, A. A. Amoscato, L. L. Pearce, J. Peterson, Metalloporphyrin $Co^{(III)}$ TMPyP Ameliorates Acute, Sublethal Cyanide Toxicity in mice. Chem. Res. Toxicol. 25 (2012) 2678-2686.

[50] J. Ma, P. K. Dasgupta, F. H. Zelder, G. R. Boss. Cobinamide chemistries for photometric cyanide determination. A merging zone liquid core waveguide cyanide analyzer using cyanoaquacobinamide. Anal.Chim.Acta, 736 (2012) 78-84.

[51] L. A. Greenawald, J. L. Snyder, N. L. Fry, M. J. Sailor, G. R. Boss, H. O. Finklea, S. Bell, Development of a cobinamide-based end-of-service-life indicator for detection of hydrogen cyanide gas. Sens. Actuators B Chem. 221 (2015) 379-385.

[52] Chan, J. Jiang, A. Fridman, L. T. Guo, G. D. Shelton, M. -T. Liu, C. Green, K. J. Haushalter, H. H. Patel, J. Lee, D. Yoon, T. Burney, D. Mukai, S. B. Mahon, M. Brenner, R. B. Pilz, G. R. Boss, Nitrocobinamide, a New Cyanide Antidote That Can Be Administered by Intramuscular Injection. J. Med. Chem. 58 (2015) 1750-1759.

[53] P. Singh and M. Kaur, CN^- scavenger: a leap towards development of a CN^- Antidote. Chem. Commun. 47 (2011) 9122-9124.

[54] P. Singh, S. Kaur, A. Singh, V. S. Mithu, A rationally designed molecule for removal of cyanide from human blood serum and cytochrome c oxidase. RSC. Adv. 4 (2014) 61884-61889.

[55] H. Nůsková, M. Vrbacký, Z. Drahota, J. Houštěk, Cyanide inhibition and pyruvate-induced recovery of cytochrome c oxidase, J. Bioenerg. Biomembr. 42 (2010) 395–403.

[56] Cytochrome c oxidase assay kit, Sigma Aldrich, product code CYTOCOX1.

[57] P. Singh, S. Kaur, J. Kaur, G. Singh, R. Bhatti, R. J. Med. Chem. 59 (2016) 3920-3934.

Highlights

- Structural and functional aspects of cytochrome c oxidase (CcOX)
- Rationally designed molecules for removal of cyanide fron CcOX
- Revival of CcOX activity

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